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Unraveling the multifunctionality of human Spindly

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# Unravelling the multifunctionality of human Spindly

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*Supervisor Dr. Eric Griffis*

Submission for the degree of  
Doctor of Philosophy

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## **Declaration**

This thesis, submitted for the degree of Doctor in Philosophy at the University of Dundee, has been performed in the laboratory of Dr. Eric Griffis at the Centre for Gene Regulation & Expression within the College of Life Sciences, Dundee. The presented work was performed under the guidance of Dr Eric Griffis and contains no material which has been accepted for the award of any degree in any university.

Claudia Conte

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I declare that Claudia Conte has spent the equivalent of at least nine terms in the research department of the College of Life Sciences at the University of Dundee and that she has fulfilled the conditions of Ordinance General No. 39 of the University of Dundee and is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Dr. Eric Griffis

*Supervisor*

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## **Abstract**

Spindly was discovered in 2007 as a protein crucial for cells to progress through mitosis. It was shown to be required for recruitment of the dynein/dynactin motor complex to kinetochores thus to promote chromosome alignment and mitotic progression. Kinetochores recruitment of the dynein/dynactin motor complex is crucial for maturation of kinetochore-microtubule attachments and for silencing the spindle assembly checkpoint (SAC), the surveillance pathway that monitors bi-orientation and inhibits anaphase onset until chromosomes are attached to opposing spindle poles. In human cells, Spindly depletion produces strong chromosome alignment defects with cells arrested in mitosis. Conversely expression of a single point mutant form allows for separation of these two functions of Spindly: it rescues chromosome alignment but inhibits SAC silencing and the recruitment of the motor complex.

In this work the interaction of Spindly with the dynein/dynactin motor complex was examined, untangling the subunits specifically involved in the binding. It was demonstrated that the single point mutation specifically impairs the interaction with dynactin, potentially affecting the capacity of the motor to strip proteins away and suggesting a role for Spindly as an adaptor of the complex, involved in enhancing its processivity.

Previous reports have shown that the constant presence of Spindly at kinetochores impedes SAC inhibition, whereas its depletion allows for an alternative mechanism dynein- independent to silence the SAC on aligned kinetochores. In the present thesis the interaction of Spindly with the proteins involved in the SAC pathway was described, hinting at a further role of Spindly in the SAC signalling related to activation/maintenance.

It has been earlier described that depletion of *Drosophila melanogaster* Spindly generates defects also in cytoskeletal organisation. Here was identified a pool of human Spindly in interphase cells localising at microtubule plus-ends. It was proved that Spindly plays a direct role in cell migration and that it localises at the leading edge of migrating cells, specifically at focal adhesion sites, together with actin filaments and dynein/dynactin.

With this work we present the discovery of new functions of human Spindly as a novel promoter of dynein/dynactin processivity in different biochemical processes where Spindly could represent a key scaffold protein necessary to link this motor complex to multiple cargos/sites.

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## List of abbreviations

3AT:	3-amino-1, 2, 4-triazole
6AAA ATPase:	ATPases Associated with diverse cellular Activities
A280:	absorbance at 280nm
ACN:	acetonitrile
APC/C:	Anaphase-Promoting complex/Cyclosome
ARP1:	actin related protein
Arp2/3:	actin-related protein-2/3
ATP:	adenosine triphosphate
BFP:	blue fluorescent proteins
BicD/ BicD2:	protein Bicaudal D
BNIP1:	BLC2/Adenovirus E1B 19 kDa Interacting Protein 1
BrdU:	bromodeoxyuridine
BSA:	bovine serum albumin
Bub:	budding uninhibited by benzimidazoles
cDNA:	complementary DNA
C-Mad2:	closed Mad2
C-terminus:	carboxyl-terminus
CAP-Gly:	cytoskeleton-associated protein glycin rich
Cdc20:	cell division cycle 20
CCAN:	Constitutive Centromere Associated Network
CDK:	cyclin-dependent kinases
CENP-E:	centromere <i>protein</i> E
CENP-F:	centromere protein E
CHAPS:	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CLIP170:	cytoplasmic linker protein 170
CPC:	chromosome passenger complex
CTRL:	control
DAPI:	2-(4-amidinophenyl)-1H -indole-6-carboxamide
DSBs:	double-strand breaks
DHC:	dynein heavy chain
DIC:	dynein intermediate chain
<i>D. mel.</i> :	<i>Drosophila melanogaster</i>
DMF:	dimethylformamide
DMSO:	dimethyl sulfoxide
Spindly:	<i>Drosophila melanogaster</i> Spindly
DNA:	deoxyribonucleic acid
DOA:	drop out amino-acid
DTT:	dithiothreitol
EB1:	end binding 1
ECL:	enhanced chemiluminescence
ECM:	extracellular matrix
EDTA:	ethylenediaminetetraacetic acid
EGTA:	ethylene glycol tetraacetic acid
EM:	electron microscopy
ER:	endoplasmic reticulum
FAK:	focal adhesion kinase
FB:	human fibroblast
Fig:	figure
FLAG IP:	FLAG- Immunoprecipitation
FKBP:	FK506 binding protein
FRB:	FKBP12-rapamycin binding
FTI:	farnesyl transferase inhibitor
Fwd:	forward

GAPDH:	Glyceraldehyde 3-phosphate dehydrogenase
GDI:	Guanosine nucleotide dissociation inhibitor
GEF:	Guanine nucleotide exchange factors
GFP:	green fluorescent protein
GM130:	<i>cis</i> -Golgi matrix 130
GTP:	Guanosine-5'-triphosphate
HI-FBS:	heat-inactivated fetal bovine serum
HIS:	histidine
HRP:	horseradish peroxidase
HSpindly:	<i>Homo Sapiens</i> Spindly
HU:	hydroxyurea
IFT:	intraflagellar transport
IgG:	immunoglobulin
IF:	intermediate filaments
IFT:	intraflagellar transport
IPTG:	Isopropyl $\beta$ -D-1-thiogalactopyranoside
IP:	immunoprecipitation
JAS:	jasplakinolide
kDa:	kilo dalton
KT:	kinetochore
JMY:	Junction-mediating and -regulatory protein
LAT A:	latrunculin A
LC:	light chain
LEU:	leucine
LiAC:	lithium acetate
LIC:	light intermediate chain
Lis1:	lissencephaly-1
LPC:	lysophosphatidylcholine
mSpindly:	<i>Mus musculus</i> Spindly
MACF:	microtubule actin cross-linking factor
Mad:	mitotic arrest deficient
MAP:	microtubule associating protein
MAPK:	Mitogen-activated protein kinases
MCC:	mitotic checkpoint complex
MEF:	mouse embryonic fibroblast
MLC:	Myosin light chain
MLCK:	Myosin light chain kinase
mM:	millimolar
$\mu$ M:	micrometer
Mps1:	MonoPolar Spindle 1
mRNA:	messenger ribonucleic acid
MS:	mass spectrometry
MT:	microtubule
MTOC:	microtubule organising centre
NAG:	neuroblastoma-amplified protein
NSF:	N-ethylmaleimide sensitive fusion protein
NudE:	nuclear distribution protein E
NudEL:	Nuclear distribution protein nudE-like
NEM:	N-Ethylmaleimide
NH <sub>4</sub> HCO <sub>3</sub> :	ammonium bicarbonate
nm:	nanometer
nM:	nanomolar
NOC:	nocodazole
N-terminus:	amino-terminus
O-Mad2:	open Mad2
PAFAH:	platelet-activating factor acetyl-hydrolase
PBS:	phosphate-buffered saline

PCNA:	proliferating cell nuclear antigen
PEG:	Polyethylene glycol
PFA:	Paraformaldehyde
p-Myosin:	phospho-Myosin
PMSF:	Phenylmethanesulfonyl Fluoride
PP1:	protein phosphatase 1
PP2A:	protein phosphatase 2 A
Rab:	Ras-related protein in brain
RanBP2:	Ran binding protein 2
RanGAP:	Ran GTPase-activating protein
Rb IgG:	rabbit Immunoglobulin
Rev:	reverse
RFP:	red fluorescent protein
RILP:	Rab-interacting lysosomal protein
RINT-1:	Rad50-interacting protein 1
RNA:	ribonucleic acid
RNAi:	RNA interference
ROCK:	Rho associated protein kinase
RPM:	revolution per minute
RZZ:	Rod-Zwilch-ZW10 complex
SAC:	spindle assembly checkpoint
SDS-PAGE:	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC:	size exclusion chromatography
siCTRL:	silencing control
siRNA:	small interfering RNA
siSpin:	silencing Spindly
SNAP:	soluble NSF attachment protein
SPDL-1:	<i>Caenorhabditis elegans</i> Spindly
Spindly IP:	Spindly immunoprecipitation
Src:	sarcoma-family kinase
ssDNA:	single strand DNA
STLC:	S-trityl-L-cystein
TBS:	Tris-buffered saline
TE:	tris-EDTA
TFA:	trifluoroacetic acid
TIRF:	total internal reflection fluorescence
TRP:	Tryptophan
UT:	untreated
WASP:	Wiskott–Aldrich syndrome protein
WAVE:	WASP-family verprolin-homologous protein
WASH:	Wiskott–Aldrich syndrome protein and SCAR homologue
X-Gal:	5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside
Y2H:	yeast-two hybrid
YFP:	yellow fluorescent protein
YPAD:	Yeast-peptone- adenine-dextrose
ZW10:	zeste-white 10

# **1 . Introduction**

## 1.1 Preamble

Animal cells possess a cytoskeletal structure that allows them to grow, divide and move, facilitating considerable changes in the cell shape. To accomplish all these functions the cytoskeleton relies on the constant interplay of three major filament systems, actin, intermediate filaments and microtubules, that have to be coordinated in order to accommodate different physiological needs. The close interplay between these cytoskeletal structures is possible thanks to different proteins, motors and cross-linkers; moreover, the strong coupling is mediated via biochemical signalling and gene regulation that allows the cytoskeletal network to cover a wide range of functions. Therefore, understanding how these pathways are organised and what are the proteins involved could help to get new insights in cell functioning.

Many cellular processes rely on the cytoskeleton network, such as cell migration and cell division. To regulate cell migration the three cytoskeletal components are highly coordinated to ensure cell polarisation and interactions with the cell matrix. Microtubules and actin filaments collaborate at the cell front to drive protrusion formation and to mediate the delivery of different components. Also, they closely interplay in cell adhesion; while the actin cytoskeleton generates traction forces to drag the cell body, microtubules control the dynamics of adhesion complexes. In a similar way, actin filaments and microtubules work together in cell division, mainly in centrosome separation and in cytokinesis, ensuring generation of two daughter cells. Central for this process is the formation of the mitotic spindle, a microtubule-based structure that allows capture and alignment of chromosomes and in turn faithful segregation.

Spindly was identified as an important molecular player of the cell division, critical to ensure proper chromosome alignment and correct transmission of the genomic material. However, its depletion was shown to affect also cytoskeletal structures in interphase cells, indicating a potential general role in the maintenance of the cytoskeleton network activities.

Work in this thesis aims to untangle different aspects of the functioning of Spindly in both mitosis and interphase, focusing on its capacity to regulate the cytoskeleton network.

## 1.2 Cell division

Prokaryotes and eukaryotes have common strategies for cell division. Although the cell organisation is much different, in both cases cells first grow and duplicate the DNA in each chromosome and then chromosomes are segregated in equal number to each daughter cell ensuring fidelity of the transmission of genome and cytoplasm.

Since prokaryotes are much simpler, cell division is faster and easier; they contain only a single circular chromosome and replication and segregation are often coupled. Chromosomes are bound to the cell membrane and thus when a cell separates each daughter will inherit the new chromosome. This process is called Binary Fission and it generates genetically identical daughter cells (Reyes-Lamothe *et al.*, 2012). In eukaryotes, the process is much more intricate, due to the higher complexity of the cell organisation. It is possible to distinguish an INTERPHASE, when the DNA duplication occurs, and a MITOTIC PHASE, when the chromosome segregation process happens. Interphase can in turn be divided in three different phases required to prepare the cell to properly transmit its genetic material once in mitosis. There are three GAP phases: **G<sub>1</sub>** (or Gap phase 1), important for the cell to grow in size and synthesise mRNA, protein and ribosomes; **G<sub>2</sub>** (or Gap phase 2), for protein synthesis and organelles replication before mitosis; **G<sub>0</sub>** (or Gap phase 0) that occurs after mitosis, in lieu of or within **G<sub>1</sub>** and frequently takes place as cells terminally differentiate and most probably will never divide again. Between **G<sub>1</sub>** and **G<sub>2</sub>** is the synthesis phase (or **S phase**) during which chromosomes are replicated to generate two sister chromatids so to double the amount of DNA. All these steps are crucial for proper initiation of the cell division process, the mitotic phase (or M phase). This can be divided into five steps:

- 1) **Prophase:** the DNA condensate into chromosomes and the MT cytoskeleton is reorganised; the centrosomes (the main microtubule organising centre in animal cells)

are replicated and move to the opposite poles thanks to the activity of motor proteins; the mitotic spindle starts to form outside the nucleus.

2) **Prometaphase:** the nuclear envelope (the physical barrier that encloses the nucleus) breaks down and sister chromatids are released from the nucleus. Sister chromatids are bound to each other at the centre thanks to the kinetochore (a multiprotein structure formed around the centromere), which allows microtubules to capture chromosomes. Microtubules generated from either end of the cell and attached to the kinetochores are now called kinetochore microtubules (k-fibres).

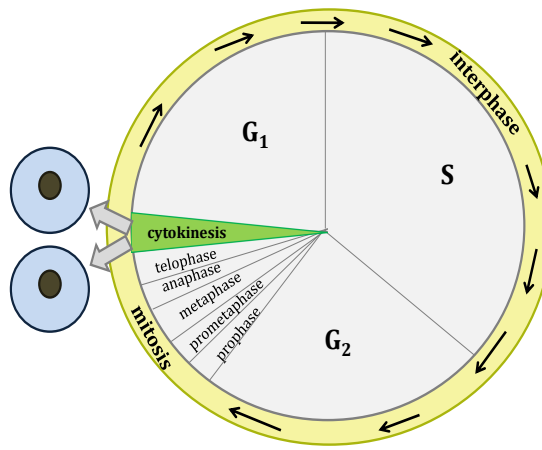
3) **Metaphase:** microtubules and kinetochores bind to each other to position chromosomes aligned at the centre of the cell and to generate the 'so-called' metaphase plate.

4) **Anaphase:** sister chromatids are separated and pulled by the spindle to the opposite poles of the cell while the spindle simultaneously elongate and move towards opposite poles. In doing so, anaphase ensures that each daughter cell receives an equal set of chromosomes.

5) **Telophase:** the nuclear envelope re-forms around each set of chromosomes, thus separating the nuclear DNA from the cytoplasm, and the chromosomes begin to decondense.

The final step of cell division is cytokinesis, characterised by the separation of the parental cytoplasm following the generation of a cleavage furrow by invagination of the plasma membrane (Fig. 1.1.) (Alberts *et al.*, 2007).





**Figure 1. 1. Schematic representation of cell division cycle.**

Cell division relies on many different steps that have to properly occur to ensure fidelity to the process: microtubules have to form a stable bipolar spindle and correct attachments have to be generated with the kinetochore site, to then allow correct alignment and separation. These steps are strictly controlled by the cell that presents an error correction mechanism to disrupt mis-attachments and a surveillance mechanism to prevent anaphase until correct inter-kinetochore tension is generated. Errors in these processes are indeed highly correlated with aneuploidy and tumorigenesis (Holland and Cleveland, 2012). Correct chromosome alignment is therefore coordinated with the silencing of the mitotic checkpoint to allow anaphase onset only upon bi-orientation. How these steps are so finely synchronised has not been fully elucidated yet. The identification of Spindly, a protein essential for both chromosome alignment and checkpoint silencing, indicates the possible presence of a ‘molecular sensor’ able to coordinate these two processes (Griffis *et al.*, 2007) (Chan *et al.*, 2009) (Barisic *et al.*, 2010) (Gassmann *et al.*, 2010).

### 1.2.1 The mitotic spindle

The whole cell division process relies on the proper movement of chromosomes; it is powered by a macromolecular machine known as the mitotic spindle apparatus, made up of centrosomes, chromosomes, microtubules (MT), cross-linkers, and microtubule motors.

The mitotic spindle assembly begins in early prophase, while the nuclear envelope is still intact. The core of the mitotic spindle is represented by microtubules, 13 parallel protofilaments of heterodimers of  $\alpha$ - and  $\beta$ - tubulin that are arranged in a hollow tube and are generated by polymerisation of tubulin subunits from a microtubule nucleating structure (or microtubule organising centre (MTOC)), which in many cells is the centrosome (Alberts *et al.*, 2007). Centrosomes are composed of two centrioles surrounded by an amorphous mass of protein called pericentriolar material (or matrix) that regulates microtubule behaviour (Woodruff *et al.*, 2014). To ensure equality in the formation of the spindle, centrosomes have to duplicate in S phase and then separate at the onset of mitosis. It is crucial for a cell to avoid the over-duplication of centrosomes, which could generate multipolar spindles and abnormal cell division. The separation of centrosomes and their migration towards the poles of the cell is mediated by microtubule forces and motor proteins like dynein and kinesin Eg5 that slide anti-parallel MTs attached to centrosomes (Hinchcliffe and Sluder, 2001).

Microtubules grow outwards from their site of polymerisation at centrosomes, extending outwards with their plus-ends polymerising to facilitate spindle organisation while the minus-end is normally bound to the MTOC. An interesting feature of microtubule growth is ‘dynamic instability’: an alternate period of slow polymerisation (elongation) followed by rapid depolymerisation (shortening). When microtubules go from growing to shortening, it is called ‘catastrophe’; conversely when the opposite occurs it is referred as ‘rescue’ (Mitchison and Kirschner, 1984).

MT dynamic is governed by the rate of GTP hydrolysis, the concentration of soluble tubulin dimers within the cell, and microtubule nucleating proteins that bind to plus-ends. Tubulin subunits bind the nucleotide GTP and only the GTP-bound tubulin can polymerise into MTs; then GTP hydrolyses to GDP rapidly after polymerisation (Desai and Mitchison, 1997). Growing microtubules contain a cap of GTP-tubulin, and the loss of this cap leads to catastrophe (Alberts *et al.*, 2007). The tubulin nucleotide exchange rate differs according to the phase the cell is in: it requires minutes in interphase, while only seconds in mitosis. During the G<sub>2</sub>/M transition the frequency of catastrophe is higher, thus microtubules are shorter and more dynamic as spindles form (Belmont *et al.*, 1990).

#### ***1.2.1.1 Mitotic spindle formation***

To date, two different theories of spindle assembly have been proposed.

The first postulated theory is based on the “search and capture” model and was described for the first time by Mitchison and Kirschner and then furtherly visualised and validated by other researchers (Mitchison and Kirschner, 1984) (Hayden J. H., 1990) (Rieder and Alexander, 1990). This model is based on centrosomal MT nucleation followed by cycles of growth, shrinkage, and regrowth to explore the cytoplasm; eventually microtubules will make contact with (or ‘capture’) the kinetochore of a chromosome, arresting its dynamics (O'Connell and Khodjakov, 2007). Further studies demonstrated that the capture timing of KT-MT is not based on a random search for a single target but there is a signal based on a Ran-GTPase gradient present around mitotic chromosomes that facilitates the capture (Wollman *et al.*, 2005). This gradient induces microtubule nucleation and stabilisation by increasing the microtubule rescue frequency and reducing the microtubule catastrophe frequency when it is located proximal to chromosomes (Carazo-Salas *et al.*, 2001). It derives from the activity of the

chromosome-associated guanine-nucleotide-exchange (GEF) factor RCC1 that binds to chromosomes and generates a high local concentration of Ran-GTP, which in turn binds to Importin- $\beta$  inducing the release of the MT stabilizing factors that it normally sequesters (Carazo-Salas *et al.*, 1999). Then RanGTP diffuses away from chromosomes before the GTP becomes hydrolysed into GDP, therefore generating a gradient that sharply decreases with distance from the chromosomes (Kalab *et al.*, 2002). In addition to stabilising microtubules to promote kinetochore capture, chromatin bound RCC1 has been shown to directly stimulate microtubule nucleation around chromatin (Carazo-Salas *et al.*, 1999). The discovery of the GTPase gradient allowed scientists to postulate the second theory for the spindle formation, useful to explain this process in those cells that do not present centrosomes, such as oocytes and *Xenopus* egg extracts (Carazo-Salas *et al.*, 2001). More precisely, this theory is based on acentrosomal nucleation of microtubules near chromosomes and their subsequent assembly into anti-parallel bundles that will adopt a spindle-like structure. It has indeed been demonstrated that microtubules can be nucleated from the kinetochores just as from the centrosomes, through a MT plus ends polymerisation process (Kitamura *et al.*, 2010). Data collected from Kitamura and colleagues also demonstrated that microtubules derived from KTs can interact with microtubules nucleated from spindle poles along their length, facilitating the loading of chromosomes onto the lateral surface of microtubules.

These two mechanisms are not mutually exclusive, but instead they look to be interconnected; indeed the ‘search and capture’ model can be applied also to those microtubules nucleated from chromosomes (Wadsworth and Khodjakov, 2004). Moreover, a common structure exists that can combine microtubules nucleated from kinetochores and microtubules nucleated from centrosomes; astral microtubules can be captured by k-fibres and the distal ends of k-fibres can then be transported poleward along astral microtubules by dynein motors (Khodjakov *et al.*, 2003). The motor

complex dynein/dynactin, as well as the Nuclear Mitotic apparatus (NuMA) protein, are involved in establishing and maintaining the standard spindle width and in crosslinking microtubules to generate sliding forces (Merdes *et al.*, 1996).

What is hence clear is that cells rely on multiple mechanisms to rapidly assemble the mitotic spindle.

### 1.2.2 The kinetochore

Proper chromosome segregation requires correct attachments between microtubules and the kinetochore sites on chromosomes. After nuclear envelope breakdown has occurred, kinetochores start to interact with the spindle microtubules; attachments are predominantly made ‘side-on’ along the microtubule lattice and then replaced by ‘‘end-on’’ attachment. Only if these connections are properly generated chromosomes can bi-orient and become aligned on the metaphase plate.

Kinetochore proteins are directly involved not only in the association with microtubules but also in the translocation of chromosomes along microtubule polymers thanks to two main motor proteins that localise at KTs: CENP-E and dynein (Kapoor *et al.*, 2006). CENP-E allows for plus-end motility along k-fibres, moving chromosome from the poles towards the equator (MT plus-ends) whereas dynein moves chromosomes towards the minus-ends of microtubules (Sharp *et al.*, 2000)

Moreover, kinetochores are central in the regulation of microtubules dynamics; kinetochore microtubules keep growing upon attachments by adding tubulin subunits at the KT-MT interface to maintain spindle length and to promote chromosome movements prior to anaphase. Cycles of depolymerisation and polymerisation at kinetochore pairs lead to the oscillations of chromosomes at the metaphase plate (Rieder and Salmon, 1998).

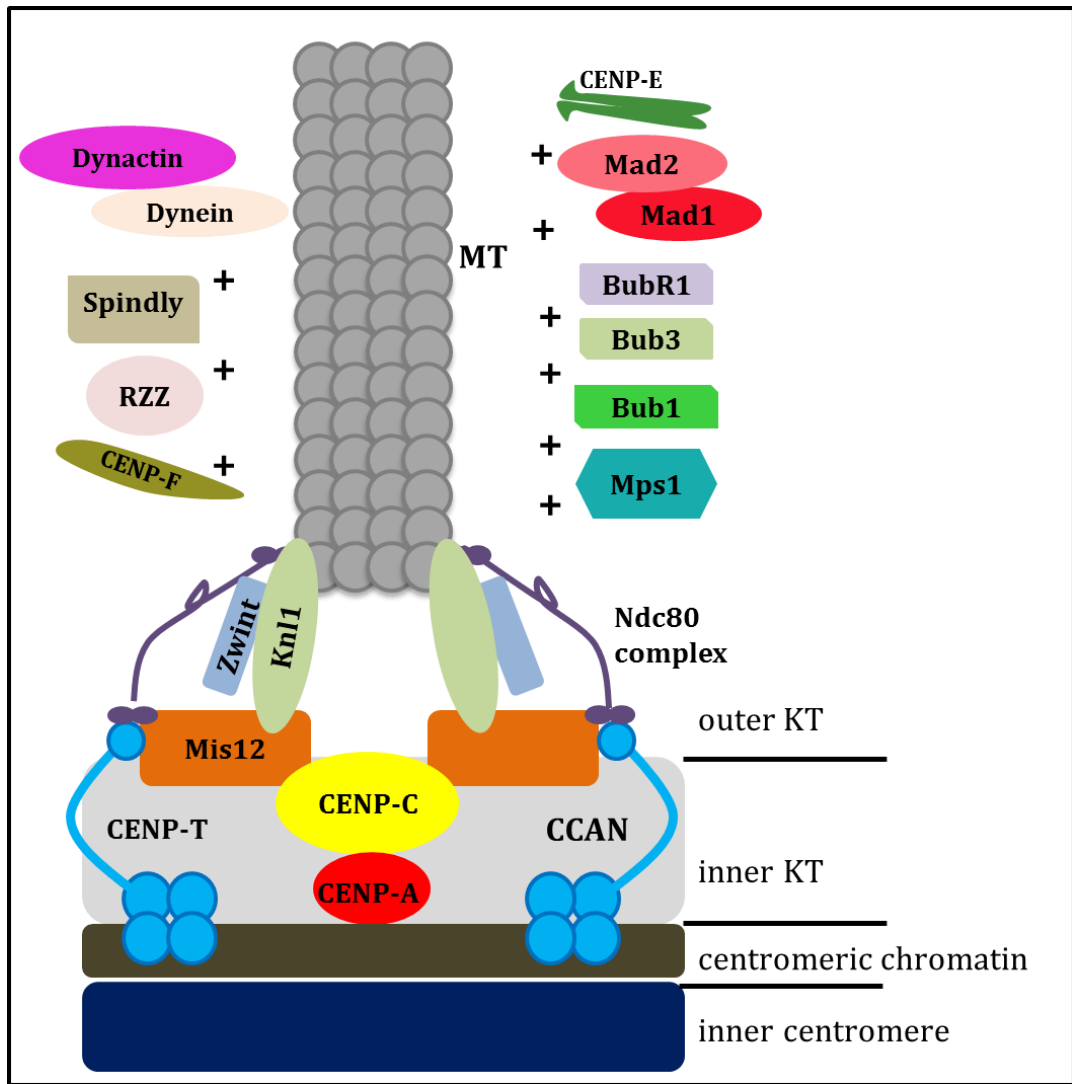
### ***1.2.2.1 Kinetochore structure***

Over the years researchers have been able to describe the ultrastructure of kinetochores, identifying three distinct regions: the inner kinetochore (which interfaces with chromatin); the outer kinetochore (which interacts with microtubules); and the central kinetochore, in between the first two (McEwen *et al.*, 1998). From the outer kinetochore the fibrous corona, a dense array of fibres, extends outwards. Each kinetochore can bind several spindle microtubules (Cheeseman, 2014).

The assembly of the kinetochore relies on the presence at the centromere of CENP-A nucleosomes, a variant of histone H3, with which associate those proteins that constitutively bind to centromeres generating the so-called ‘constitutive centromere-associated network’, or CCAN. This network of proteins helps to define the ‘kinetochore-restriction site’ where MT attachments are made (McAinsh and Meraldi, 2011). All the other proteins that assemble in the kinetochore are recruited between the end of G<sub>2</sub> phase and the beginning of M phase. Some of them are quite dynamic and so are depleted following microtubule attachments; others persist longer, until the end of mitosis or even until G<sub>1</sub> phase (Liu *et al.*, 2006).

Cheeseman and co-workers identified ten proteins central for the kinetochore-microtubule interaction; they constitute the KMN network that in vertebrates is made up of the Knl1 complex (Knl1 and Zwint), the Mis12 complex (Nnf1, Mis12, Dsn1 and Nsl1) and the Ndc80 complex (Ndc80 (Hec1), Nuf2, Spc24 and Spc25) (Cheeseman *et al.*, 2006). The KMN network serves as an important kinetochore ‘hub’ for both microtubule attachments and spindle checkpoint assembly and signalling. The Ndc80 complex and the Knl1 complex are crucial respectively to bind microtubules and stabilise KT-MT associations.

Moreover they can mediate the targeting of spindle assembly checkpoint proteins onto kinetochores (Varma *et al.*, 2013): by recruiting Bub1, BubR1, Bub3, PP1, Mps1 and the Rod-Zwilch-ZW10 (RZZ) complex via the Knl1 complex (Vleugel *et al.*, 2013) (Espeut *et al.*, 2012) (London *et al.*, 2012) (Starr *et al.*, 2000), and by mediating the association of Mps1 and of the Mad1-Mad2 complex through the Ndc80 complex (Zhu *et al.*, 2013) (Martin-Lluesma *et al.*, 2002). Additionally, Ndc80 links the CCAN with the outer kinetochore plate by binding CENP-T (Nishino *et al.*, 2013). The third component of the KMN complex is the Mis12 complex that interacts with CENP-C on one side and with the Ndc80 complex and the Knl1 complex on the other side bridging in this way the outer kinetochore region and the inner centromeric DNA (Screpanti *et al.*, 2011) (Kline *et al.*, 2006) (Fig. 1.2).



**Figure 1. 2. Schematic representation of the kinetochore structure.**

Molecular architecture of a kinetochore showing (from the bottom to the top): the inner centromere, the centromeric chromatin, on which assembles the CCAN network of proteins forming the inner KT, and the outer KT. The CCAN recruits then the KMN network (Knl1/Mis12/Ndc80 complexes) that operate in the formation of outer KT plate, thus to recruit other molecular players for execution of mitosis: Bub1, Bub3, BubR1, Mad1/Mad2, Mps1, RZZ, Spindly, dynein/dynactin (Suzuki *et al.*, 2015) (Petrovic *et al.*, 2010) (Vos *et al.*, 2011) (Zhang *et al.*, 2015) (Kim *et al.*, 2012) (Vleugel *et al.*, 2013) (Moyle *et al.*, 2014).

#### 1.2.2.2 Chromosome-microtubule attachment

Errors in the chromosome partitioning process generate chromosome aberrations and formation of malignancies (Holland and Cleveland, 2012). These defects can be related to different aspects of cell division: a weak mitotic checkpoint (which allows anaphase onset before all chromosomes are properly aligned on the metaphase plate), overexpression of Separase or Securin (two regulators of chromosome cohesion),



formation of multipolar spindles (which often originates from supernumerary centrosomes), and/or failure in the specification of the kinetochore formation site (Weaver and Cleveland, 2005). This latter is quite critical; failure in the specification process will impair attachments on the mitotic spindle, while definition of multiple sites will give rise to inappropriate attachments.

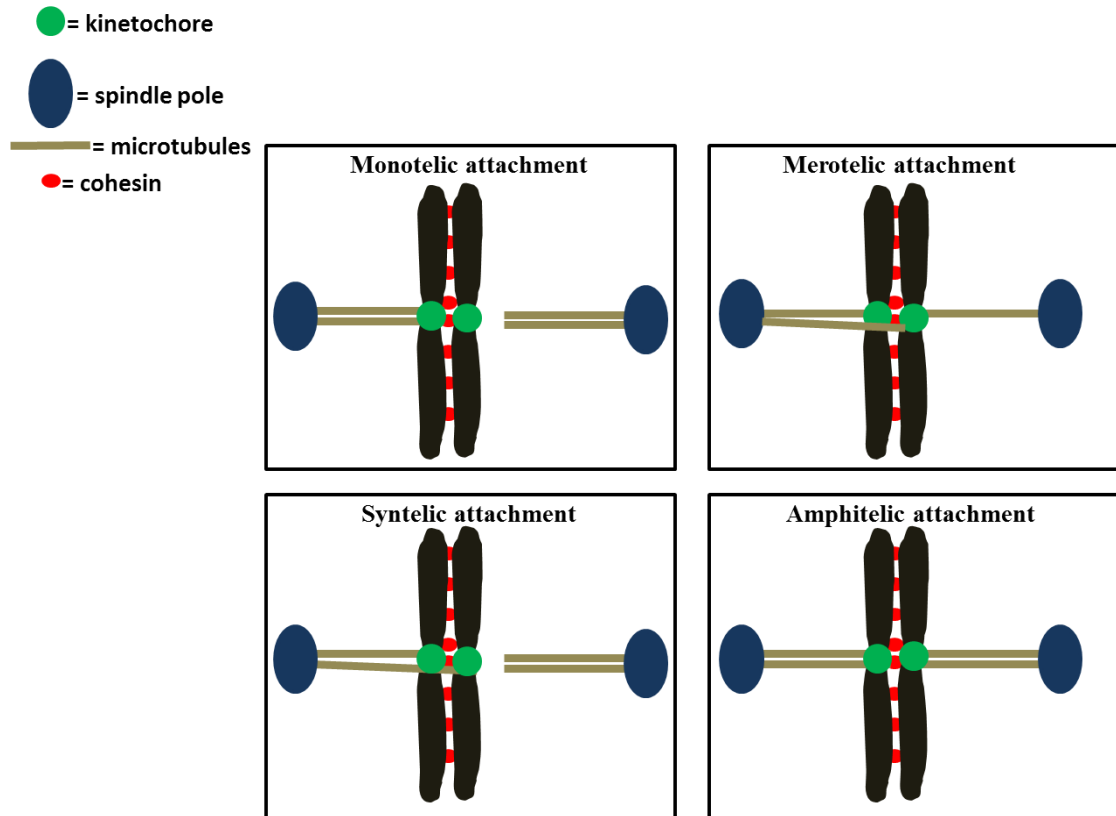
Several kinetochore binding proteins are involved in the regulation of KT-MT attachments. The whole KMN complex is essential for the microtubule interaction: the Ndc80/Nuf2 dimer, within the Ndc80 complex, and the Knl1 protein, within the Knl1 complex, present microtubule-binding sites (one each) (Cheeseman *et al.*, 2006).

These attachments are regulated by Aurora B kinase that phosphorylates microtubule binding sites, inducing detachment when improper interactions have occurred (DeLuca *et al.*, 2006). Other kinetochore proteins are involved in the generation and stabilisation of stable KT-MT attachments. The RZZ complex, recruited at KTs by Zwint (in the inner kinetochore plate), is directly involved in the association of the dynein/dynactin motor complex and it can delay microtubules 'end-on' attachments by binding the Ndc80 tail and consequently affecting the microtubule interaction (Kops *et al.*, 2005) (Gassmann *et al.*, 2008) (Cheerambathur *et al.*, 2013). In a similar way, inhibition of Spindly, another important player in the kinetochore recruitment of the dynein/dynactin motor complex, has been shown to generate defects in chromosome segregation resembling the lack of 'end-on' attachments (Gassmann *et al.*, 2008) (Gassmann *et al.*, 2010).

Dynein is a promoter of load-bearing attachments; only once associated with KTs, it can accelerate their formation and at the same time it can avoid the generation of improper attachments providing a force that orients the KT toward the spindle pole at which the particular microtubule originates (Yang *et al.*, 2007). By doing so, dynein

reduces the possibility that the same kinetochore captures a microtubule from the opposite pole (Varma *et al.*, 2008).

Although many proteins control the process of attachments, chromosomes can engage in a variety of erroneous bindings besides the correct amphitelic (bi-oriented) one. Three major types of mis-attachment can occur: **merotelic attachments**, where one kinetochore binds to microtubules from both poles; **syntelic attachments**, in which both kinetochores have bound microtubules from the same pole; **monotelic attachment**, where one kinetochore attaches to microtubules from one spindle pole and the other kinetochore is not bound (Fig. 1.3) (Tanaka, 2008).



**Figure 1. 3. Kinetochore-microtubules interactions.**

Schematic representation of the possible attachments that can occur in mitosis between KT and MTs. Monotelic and syntelic attachments generate mono-orientated mitotic spindles (that is connected to only one spindle pole) while merotelic and amphitelic attachments give rise to bi-orientated mitotic spindle (connected to both poles) (for full description see the main text).

The frequency of mis-attachments can be high within a cell; therefore they have to be either prevented or corrected. Cells possess an error-correction pathway that allows faithful chromosome segregation to occur even when mis-attachments have been made. This signalling is mediated by Aurora B kinase and detects and corrects mono-orientated attachments (Lampson et al., 2004). Aurora B localises between the sister kinetochores where it can phosphorylate the Ndc80 complex and consequently reduce its microtubule binding affinity (Ciferri *et al.*, 2008). Therefore, Aurora B keeps up the phosphorylation status of Ndc80 until proper tension is not generated (upon correct attachments). Once bi-orientation has occurred, tension is produced and kinetochores are pulled apart, physically separating Ndc80 from centromeric Aurora B.

Two important binding partners of Aurora B are Survivin and INCENP, which are positioned between microtubules and kinetochores and so are able to sense the tension generated upon bi-orientation (Lampson and Cheeseman, 2011). These four proteins (Aurora B, Borealin, Survivin and INCENP) function together in the same complex, the chromosome passenger complex (CPC) that is involved not only in the error-corrections process but also in the regulation of bipolar spindle stability and completion of cytokinesis (Carmena *et al.*, 2012). The CPC re-localises throughout mitosis to ensure phosphorylation of different substrates and so the regulation of different pathways during cell division. The CPC is essential also for spindle checkpoint activation and maintenance promoting the recruitment of Mps1 to KTs (Saurin *et al.*, 2011). Consequently Aurora B kinetochore substrates are de-phosphorylated to allow mitotic progression; its counteracting phosphatases are protein phosphatase 1 (PP1), at the outer kinetochore, and protein phosphatase 2 A (PP2A) B56 subunit, at the inner centromere (Funabiki and Wynne, 2013).

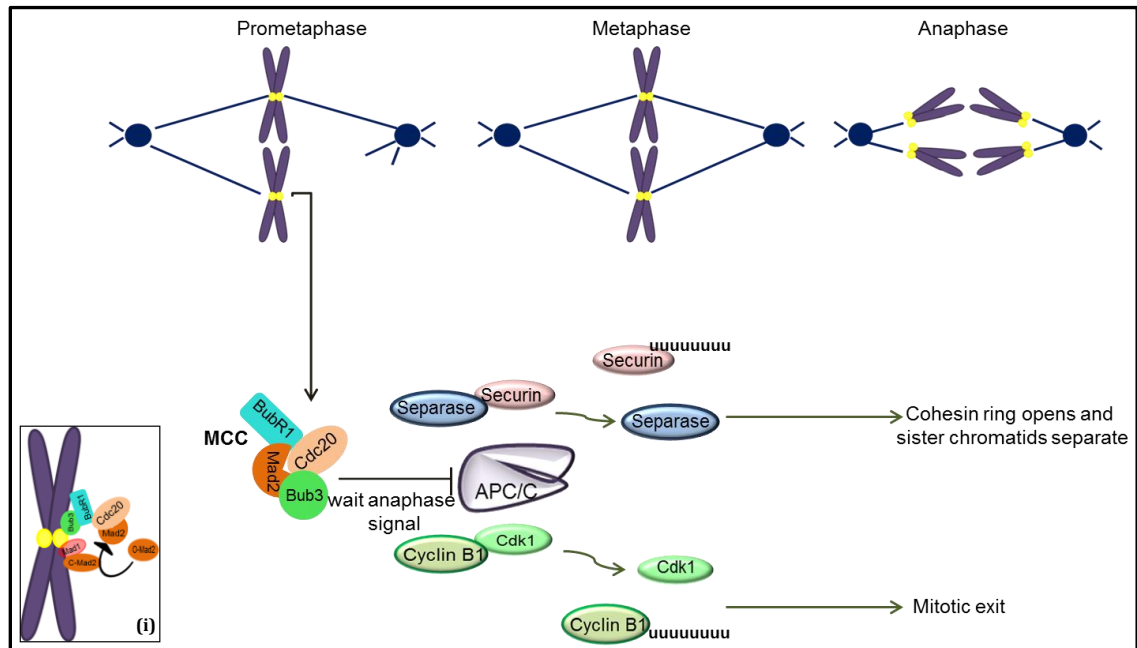
### 1.2.3 The spindle assembly checkpoint

Cells utilise a surveillance and signalling system that monitors MT-KT interactions and delays anaphase onset until all chromosomes are properly attached to the spindle. This checkpoint, called the Spindle Assembly Checkpoint (SAC), has to guard against the degradation of Cyclin B and the cleavage of the cohesin rings that keep duplicated chromosomes (sister chromatids) linked from the moment they are replicated until anaphase onset (Nasmyth and Haering, 2009). Once anaphase starts, the cohesin-ring is physically cleaved by Separase and by spindle forces that pull chromatids towards the spindle poles. This means that until the last kinetochore is correctly attached sister chromatids have to be linked to resist pulling forces from the spindle and to prevent early chromosome segregation. Additionally, for complete exit from mitosis, cells have to degrade Cyclin B as well (Sivakumar and Gorbsky, 2015). Therefore the mitotic checkpoint sets the time when mitotic exit and anaphase onset can occur (Musacchio, 2011) (Lara-Gonzalez *et al.*, 2012).

The molecular players involved in this signalling were identified in 1991 in a genetic screen in *Saccharomyces cerevisiae* that looked for mutants that failed to arrest in mitosis after microtubule destabilisation. The screens identified Mad1, Mad2, Mad3 (mitotic arrest-deficient), Bub1, and Bub3 (binding uninhibited by benzimidazole) (Hoyt *et al.*, 1991) (Li and Murray, 1991). Subsequently homologues were described in higher eukaryotes and the kinetochore kinase Mps1 (monopolar spindle) with a dominant effect on the checkpoint was described (Weiss and Winey, 1996). Initially, how the complex was functioning was not clear; to fully understand the mechanism more complicated cell biology experiments were performed utilising laser ablation and physical manipulation of chromosomes. These experiments guided researchers attention first to kinetochores and hence to the importance of tension generation upon MT-KT attachment (Rieder *et al.*, 1995).

SAC proteins are recruited on unattached KT's and are removed only upon kinetochore stretching that indicates correct bi-orientation (Uchida *et al.*, 2009) (Maresca and Salmon, 2010). The core SAC effector is the mitotic-checkpoint complex (MCC), made up of Mad2-Cdc20 and BubR1-Bub3 (Chao *et al.*, 2012) (Fig. 1.4, (i)). Additional proteins involved in this signalling are the RZZ complex, p31<sup>comet</sup>, kinases including Aurora B, Mps1, MAPKs, Cdk1-Cyclin B and Plk1, the motor proteins CENP-E and dynein, and dynein-accessory factors (dynactin, NudE/L, Lis1, Spindly) (Karess, 2005) (Eytan *et al.*, 2013) (Takenaka *et al.*, 1998) (D'Angiolella *et al.*, 2003) (Mao *et al.*, 2003) (Silva *et al.*, 2014).

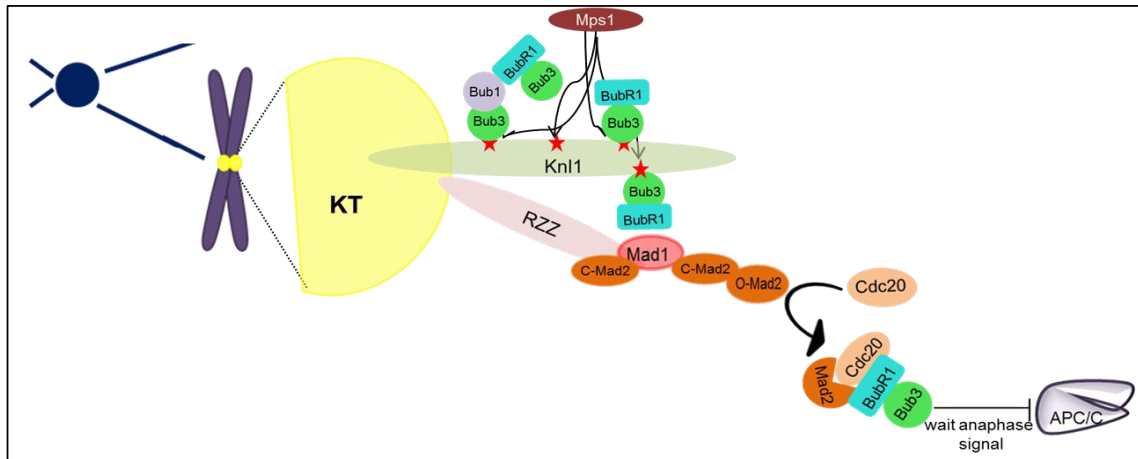
The SAC functions generating a “wait anaphase” signal that inhibits the E3 ubiquitin ligase APC/C (anaphase-promoting complex/cyclosome) impairing proteasomal degradation of two crucial substrates, Cyclin B, the mitotic Cdk1 cofactor, and Securin, the protein that protects sister chromatid Cohesin from Separase (Peters, 2006). Inhibition of APC/C is directly mediated by the MCC complex that binds Cdc20 through Mad2 blocking APC/C-Cdc20 (Fang *et al.*, 1998); association of BubR1 also potentiates the inhibitory signal Mad2-mediated, reinforcing the idea that is the whole MCC complex necessary to prevent anaphase onset (Fang, 2002), (Fig. 1.4).



**Figure 1. 4. Schematic representation of the regulation of the spindle assembly checkpoint.** In prometaphase is catalysed the assembly of the mitotic checkpoint complex (MCC; made up of Bub3/BubR1/Mad2/Cdc20) onto unattached kinetochores. This complex inhibits the APC/C ubiquitin ligase. Once bi-orientation has been achieved (metaphase), the MCC is not assembled any longer and the APC/C is activated and promotes the degradation of Securin and Cyclin B1 and in turn sister chromatids separation and inactivation of Cdk1 (anaphase). These steps will allow for mitotic exit. (i) Model of SAC effectors assembly onto unattached KT's.

#### 1.2.3.1 Spindle Assembly Checkpoint formation

SAC molecular players get associated to kinetochores in prometaphase through Bub1, a fundamental SAC kinase recruited at first via the Knl1 complex (Sharp-Baker and Chen, 2001) (Johnson *et al.*, 2004). Knl1 specifically interacts with Bub1 upon Mps1 phosphorylation of its *N*-terminus (Kiyomitsu *et al.*, 2011) (London *et al.*, 2012). Phospho-Knl1 also allows the localisation of BubR1, a pseudo-kinase important for chromosome bi-orientation, and of Bub3, a further component of the MCC. While Bub1 kinetochore localisation depends on Bub3 interaction with Knl1, BubR1 localisation relies on Bub1, suggesting a dimerisation between these two proteins (Taylor *et al.*, 1998) (Vleugel *et al.*, 2015) (Fig. 1.5).



**Figure 1. 5. Diagram representing spindle assembly checkpoint formation pathway.**

Mps1 phosphorylates multiple sites on Knl1 (red stars) allowing the subsequent association of Bub3 and binding of Bub1 and BubR1. This first step is critical to then promote recruitment of other SAC components to activate the “wait anaphase signal” and inhibits APC/C (for detail see main text).

Bub1 is also central for the sequential localisation of Mad1 at KT, which then remains stably associated with KT until microtubules have attached (Howell *et al.*, 2004). The recruitment of Mad1 to kinetochore occurs at the beginning of prometaphase and it allows for further association of Mad2 to site. It has been demonstrated that Mad2 exists in two conformations: a closed conformation (C-Mad2) that is bound to Mad1, and an open conformation (O-Mad2) that is present in the cytoplasm and that is transiently engaged with the kinetochore (Vink *et al.*, 2006) (De Antoni *et al.*, 2005). Precisely, O-Mad2 dimerises with C-Mad2 already presents at KT and is then converted into C-Mad2 that binds to Cdc20, making up a complex that will allow for subsequent assembly and functioning of the MCC (De Antoni *et al.*, 2005). Stable kinetochore localisation of Mad1 required also the RZZ complex that is first engaged at KT upon Aurora B-mediated phosphorylation of Zwint, a constitutive binding protein of the Knl1 complex (Fig. 1.5) (Kasuboski *et al.*, 2011) (Varma *et al.*, 2013).



### ***1.2.3.2 Spindle Assembly Checkpoint silencing***

The correct chromosome alignment and thus the correct attachments between KTs and MTs induce the inactivation of the SAC. There are different theories that try to explain this silencing process; they seem to be all somehow coordinated and therefore strictly related to one another.

#### **The stripping model**

The physical removal, or “stripping”, of checkpoint proteins away from the kinetochore is essential for silencing the SAC. It has indeed been shown that when Mad1 is artificially tethered to kinetochores (via the KMN component Mis12), Mad2 is continuously captured and cells are arrested in metaphase; moreover, by increasing the levels of Mad1 at bioriented KTs upon SAC silencing it is possible to reactivate the signalling indicating that all the SAC molecular players have to be removed from KTs to allow progression through mitosis (Ballister *et al.*, 2014).

The transport of the SAC components towards spindle poles depends on lateral interaction of KTs with non-kinetochore microtubules as well as with kinetochore microtubules and it is quite rapid (Howell *et al.*, 2001). This process relies on the minus-end motor complex dynein/dynactin: experiments based on ATP depletion or inhibition of dynein/dynactin by microinjection of antibodies, either anti-p50 (or dynamitin, a dynactin subunit) antibody or anti-DIC (dynein intermediate chain), have demonstrated to block this transport process and to arrest cells in metaphase accumulating Mad2 at metaphase kinetochores (Howell *et al.*, 2001). Furthermore, inhibition of the motor complex reduces generation of tension at kinetochores upon chromosome alignment, suggesting a crucial role for the motor in the process of tension generation linked as well to the activation of the SAC-silencing process (Yang *et al.*, 2007). Kinetochore recruitment of dynein/dynactin relies on the presence of the RZZ

complex and Spindly at KTs. It has been shown that depletion of Spindly not only impairs dynein recruitment at KTs but also halt cells in metaphase retaining Mad2 at high levels (Griffis *et al.*, 2007). In human cells depleted of Spindly, Mad2 can still be removed from the few aligned KTs, suggesting the presence of an alternative dynein-independent mechanism and proposing that KT-dynein-mediated removal is required only upon association of Spindly with KTs (Chan *et al.*, 2009) (Gassmann *et al.*, 2010).

#### The phosphatase-based model

The recruitment of SAC proteins to kinetochores is promoted by phosphorylation of Knl1 mediated by two kinases: Mps1 and Aurora B (Yamagishi *et al.*, 2012). Vice versa, the silencing of the mitotic checkpoint requires de-phosphorylation of those kinetochore proteins to allow removal from this site (Funabiki and Wynne, 2013). As Mps1 and Aurora B are involved in both chromosome alignment and SAC activation, kinetochore phosphatases can interplay for stabilisation of the attachments and SAC silencing. On one side stabilisation of the attachments is achieved by physical separation of Aurora B from its substrates, as a result of inter- and intra-kinetochore tension and by Mps1 phosphorylation that promotes the formation of stable ‘end-on’ attachments upon a conformational change that releases Ndc80 (Liu *et al.*, 2009) (Dou *et al.*, 2015); on the other side, phosphatases have to be recruited to KTs to ensure the stabilisation of KT-MT binding and de-phosphorylation (and subsequent release) of spindle assembly checkpoint proteins (Funabiki and Wynne, 2013). Therefore, spatial separation upon tension is not sufficient to allow prometaphase-metaphase transition.

Kinetochore phosphatases recruited for mitotic transition are protein phosphatase 1 (PP1) and protein phosphatase 2 A (PP2A). In yeast it has been shown that PP1 antagonises both Mps1 and Aurora B, while in mammalian cells this function requires kinetochore localisation of PP2A phosphatase (London *et al.*, 2012) (Kruse *et al.*, 2013)

(Espert *et al.*, 2014) (Nijenhuis *et al.*, 2014). PP1 is a serine/threonine phosphatase that controls many pathways within a cell with high specificity for each function thanks to precise interactions between the different regulatory subunits and the catalytic subunit (Korrodi-Gregorio *et al.*, 2014). Most interactions between PP1 and its binding partners occur via a RVxF site (K/R/H/N/S-V/I/L-x-F/W/Y, where x is any residue other than Phe, Ile, Met, Tyr, Asp or Pro), which allows PP1 to come close to the interactor protein to then make additional bindings. The association of PP1 with kinetochores occurs via a RVSF site present on Knl1 and once there PP1 promotes the removal of phosphate groups from Aurora B-kinetochore substrates (such as H3, Sds22, Knl1 and CENP-E) and it stabilises microtubule attachments (Liu *et al.*, 2010). However, Knl1 is also phosphorylated by Mps1 in order to recruit Bub1, Bub3 and BubR1 onto unattached KTs. It has been shown that in yeast overexpression of Mps1 enhances this binding while overexpression of PP1 directly inhibits it (London *et al.*, 2012). Thus PP1 is a key player in the mitotic exit process and it is required for inactivation of the spindle checkpoint,

Conversely, in mammalian cells there exists another process to change the phosphorylation status of kinetochores. Previous works have described a role for PP2A (specifically the B56 subunit) in the de-phosphorylation process of Aurora B-kinetochore substrates upon BubR1 recruitment (Kruse *et al.*, 2013). PP2A holoenzymes consist of a catalytic subunit (C), a scaffold subunit (A) and a regulatory subunits B (for which exists four different families). B55 and B56 are the most relevant regulatory subunits that play a role in mitosis progression (Kiely and Kiely, 2015). PP2A-B56 has been proved to be involved in different mitotic functions: sister chromatid cohesion, KT-MT attachments and chromosome movements (Kiely and Kiely, 2015). Recently its potential role in SAC silencing has been investigated and it has been shown that it is strategic for the de-phosphorylation of the Knl1 sites involved

in the kinetochore recruitment of Bub1, Bub3 and BubR1 counteracting both kinases. In this way it controls SAC silencing as well as MT-KT attachments (Espert *et al.*, 2014). In mammals is believed that these two phosphatases play a coordinated role: PP2A B56 is responsible for the initial removal of Bub proteins from KT's and then PP1 takes over to finish up the de-phosphorylation process (Espert *et al.*, 2014). Therefore, it is hypothesised a first weak inhibition signal PP2A-B56 mediated that rapidly silences the checkpoint by de-phosphorylation of Knl1 (at this point SAC can still rapidly reactivate if attachments are lost upon re-localisation of Aurora B and subsequent re-phosphorylation of Knl1), and then a complete switch off mediated by PP1 that de-phosphorylates further Knl1, bringing the kinetochore back to the initial status.

#### The disassembly model

As previously stated, the core complex of the mitotic checkpoint is represented by the MCC complex (Sudakin *et al.*, 2001). Its disassembly is key for SAC silencing. The process is mediated by p31<sup>comet</sup>, a binding partner of C-Mad2 that co-expresses with Mad2 during the cell cycle and it is needed for efficient progression through mitosis (Habu *et al.*, 2002). p31<sup>comet</sup> recognises the same binding site as O-Mad2 and reduces the interaction between Cdc20 and Mad2 dissociating the MCC complex and preventing its re-formation (Teichner *et al.*, 2011). Such a process requires energy that is provided by the TRIP13 ATPase, a binding partner of p31<sup>comet</sup>, able to mediate the disassembly and the release of Mad2 from the MCC (Eytan *et al.*, 2014). TRIP13 also promotes the release of Cdc20 from BubR1 but the mechanism behind this process is still uncertain (Eytan *et al.*, 2014). During SAC silencing APC15 is activated as well to mediate Cdc20-auto-ubiquitination promoting the disassembly of the MCC complex (Foster and Morgan, 2012). CEUDC2, a mitotic substrate of CDK, can also bind to phospho-Cdc20 promoting Mad2 release and consequent activation of APC/C<sup>Cdc20</sup> (Gao *et al.*, 2011).

Therefore there are multiple mechanisms that induce SAC silencing; the three models just described are not mutually exclusive but they closely collaborate to drive rapid SAC inactivation and anaphase onset (Wang *et al.*, 2014).

### 1.3 Spindly

The inactivation of the SAC requires interplay of different proteins once bi-orientation has been achieved. Recruitment of the dynein motor is one of the most important steps. In this process there are different proteins involved, such as dynactin, the RZZ complex, Lis1/NudE/NudEL and Spindly (Schroer, 2004) (Starr *et al.*, 1998) (Karess, 2005) (Li *et al.*, 2005) (Griffis *et al.*, 2007).

Spindly was identified in *Drosophila melanogaster* (*D. mel.*) S2 cells (Spindly) through two RNAi screens, which looked at the mitotic index and at the spreading and morphology of these cells (Griffis *et al.*, 2007). From the interphase screening it was reported an alteration in the normal shape of S2 cells: they were not round but showed spiky and elongated microtubule-rich projections (Griffis *et al.*, 2007). The phenotype registered at mitosis was much stronger. Spindly depleted cells could not progress through mitosis but they arrested in metaphase, retaining Mad2 and Rod on aligned KT and exhibiting chromosome scattering phenotypes (Griffis *et al.*, 2007) (Gassmann *et al.*, 2010). This phenotype is perfectly in line with defects observed upon depletion of dynein (or inhibition of other previously identified dynein co-factors) (Yang *et al.*, 2007) (Varma *et al.*, 2008). After its initial description in *D.mel.*, homologues of Spindly were identified also in *C. elegans* (SPDL-1) (Gassmann *et al.*, 2008) (Yamamoto *et al.*, 2008), *H. sapiens* (hSpindly) (Griffis *et al.*, 2007) (Chan *et al.*, 2009) and *M. musculus* (mSpindly) (Zhang *et al.*, 2010), with each protein showing equivalent phenotypes when inhibited.

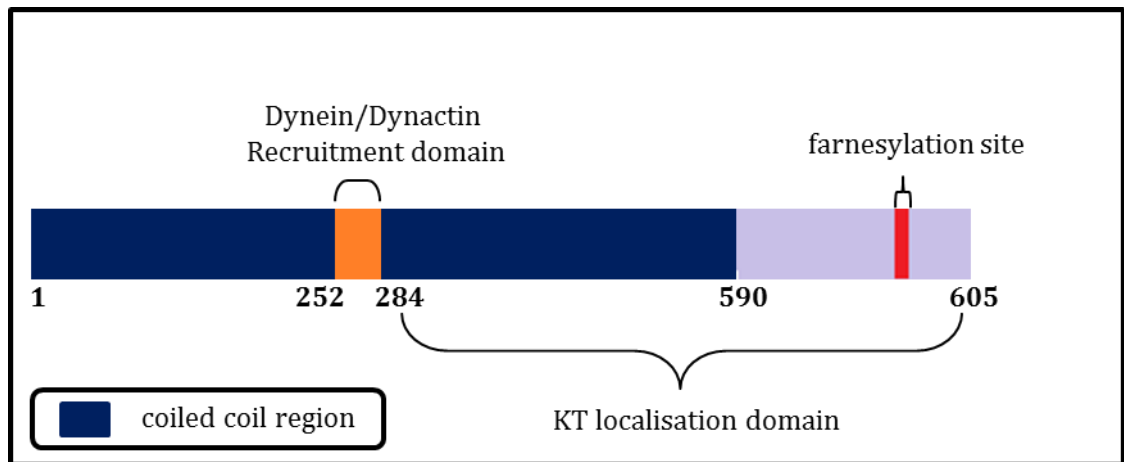
In mitosis, Spindly behaves as a classical KT protein: it localises to kinetochores in prometaphase and then, once attachments have been made, it is transported down the mitotic spindle to the spindle poles (Griffis *et al.*, 2007) (Chan *et al.*, 2009) (Gassmann *et al.*, 2010). The KT-shedding of Spindly was reported to be dynein-dependent;

depletion of the motor protein led to accumulation of Spindly on aligned kinetochores (Griffis *et al.*, 2007) (Chan *et al.*, 2009) (Barisic *et al.*, 2010). These data confirmed the interdependence between Spindly and dynein, a behaviour resembling other SAC components (Howell *et al.*, 2001).

Regarding Spindly recruitment at KTs, immunofluorescence and live imaging experiments have revealed a close relation with the RZZ complex; depletion of this complex was proved to prevent Spindly targeting to KTs, demonstrating the recruitment-dependency of Spindly for the RZZ complex and suggesting to be part of the same complex (Griffis *et al.*, 2007) (Gassmann *et al.*, 2008) (Chan *et al.*, 2009). As registered for RZZ depleted cells, Spindly depleted cells spend more time in mitosis (longer times between nuclear envelope breakdown and anaphase onset) compared to control cells. These cells show great difficulty in aligning their chromosomes, indicating that Spindly, besides its role as a kinetochore-recruitment factor for dynein/dynactin, plays a role in regulating the chromosome alignment process. Normally, indeed, the RZZ complex inhibits chromosome alignment by impairing the Ndc80 binding affinity for ‘end-on’ interactions; the presence of Spindly helps therefore to relieve this impairment by bridging RZZ with dynein and releasing Ndc80 to generate stable attachments between KTs and MTs (Gassmann *et al.*, 2008). Additionally, inhibition of Spindly expression was reported to regulate the steady state of a component of the RZZ complex, ZW10, controlling its turnover at kinetochores (Barisic *et al.*, 2010). Thus, Spindly functions as an important adaptor between the RZZ complex and the dynein/dynactin motor complex.

The human Spindly (hSpindly) gene encodes for a 605-amino acid protein consisting of two coiled coil domains separated by a short conserved stretch of amino acids, called the ‘Spindly box’ (251-KGNSLFAEV-260) (important for the recruitment

of dynein/dynactin motor complex at KTs (Gassmann *et al.*, 2010)). The C-terminus of Spindly is a highly unstructured and basic domain with a farnesylation site that allows for kinetochore localisation (Moudgil *et al.*, 2015) (Fig. 1.6).



**Figure 1. 6. Schematic representation of human Spindly domains.**

Studies revealed that a single point mutation within the ‘Spindly box’ region (either a serine or a phenylalanine into alanine: S256A or F258A for human Spindly) forms a protein that can still localise to KTs and promotes chromosome alignment but that cannot recruit the dynein/dynactin motor complex, inhibiting poleward transport of SAC proteins (Gassmann *et al.*, 2010). Curiously, cells exclusively expressing Spindly mutants have high levels of Mad1, Mad2 and CENP-E on aligned KTs, very different from what registered in Spindly-depleted cells, where SAC proteins can leave KTs in a dynein-independent manner. This suggests on one side the presence of a dynein-independent mechanism that allows for removal of SAC components from KTs and that is active only when Spindly does not localise to KTs; on the other side, the previous result indicates that if Spindly is retained at kinetochores the checkpoint is kept active (Chan *et al.*, 2009) (Gassmann *et al.*, 2010).

A further proof of this relationship between Spindly and the SAC activity came from studies conducted from Barisic and co-workers; they showed that expression of Spindly mutant constructs (lacking either the whole ‘Spindly-box’ or the first 253



amino acid of the protein) blocked SAC inactivation upon microtubule attachment and bi-orientation. Expression of these Spindly mutants also impaired the recruitment of dynein/dynactin at KTs and consequently the streaming of SAC proteins (Barisic *et al.*, 2010).

Recently, Spindly-kinetochore localisation has been deeply analysed; it has been reported that it directly depends on a sequence in the C-terminal portion of the protein that contains a variant of the CAAX box (-CPQQ-) needed for farnesylation (Moudgil *et al.*, 2015) (Holland *et al.*, 2015). In the farnesylation modification the C-terminal cysteine accepts a farnesyl group becoming more hydrophobic and increasing therefore its binding affinity for plasma membranes and proteins (Zhang and Casey, 1996). Inhibition of farnesylation (by a specific farnesyl transferase inhibitor, FTI) or mutation in the C-terminus of Spindly, showed similar reduction of Spindly kinetochore-levels and consequently a decrease in the kinetochore levels of both dynein and dynactin with an additional chromosome misalignment (Holland *et al.*, 2015). This indicates that farnesylation is involved in the interaction between Spindly and the RZZ complex (its kinetochore binding partner); to confirm this, samples pre-treated with a FTI showed reduction in the association between Spindly and ZW10 or Rod (Moudgil *et al.*, 2015). This suggests that farnesylation induces a conformational change in Spindly that promotes the interaction with the RZZ complex. Interestingly, Spindly depletion causes cells to spend much more time in metaphase when compared with FTI-treated cells, suggesting that either there is another way to silence the checkpoint that does not requires the kinetochore localisation of Spindly or that there is a compensatory mechanism that is activated in cells treated with FTI (Holland *et al.*, 2015).

Finally, Spindly has also been studied in meiosis. In mouse oocytes Spindly was shown to have the same kinetochore localisation as in mitosis (aggregating in

prometaphase) and to translocate to microtubules and to the spindle poles upon attachments. Mouse oocytes depleted of Spindly show a similar phenotype to mitotic Spindly-depleted cells, with metaphase stage arrest, indicating that Spindly participates in SAC silencing, chromosome alignment and spindle formation in meiosis as well (Zhang *et al.*, 2010).

## 1.4 Dynein

In eukaryotic cells, transport of cellular components along the cytoskeletal tracks is carried out by motor proteins, myosins, which walk along or slide on actin filaments, and kinesins and dyneins that walk on microtubules (Wickstead and Gull, 2011). Microtubule motors can be distinguished in metazoans since kinesins move towards the plus-ends (generally arrayed at the cell periphery) and dynein moves towards the minus-ends (mostly towards the MTOC). For all of these motor proteins, ATP hydrolysis is fundamental to power movement along their tracks although they have different modes of coupling ATP hydrolysis to stepping (Vale, 2000).

In vertebrates there are nine dynein subfamilies; most of them are axonemal and slide microtubules past each other to generate the beating motion of cilia and flagella, whereas only two are cytoplasmic and transport cargos along microtubules: intraflagellar transport (IFT) dynein or dynein 2, and cytoplasmic dynein or dynein 1 (Allan, 2011). Dynein 2 expression is limited to ciliated cells and within the Golgi apparatus (Mikami *et al.*, 2002); conversely dynein 1 has many different functions for all the minus-end directed transport throughout the cytoplasm in all phases of the cell cycle. Transport of organelles, mRNA and proteins, mitotic spindle orientation, nuclear and cellular migration, and chromosome movements are all dynein-dependent processes (Allan, 2011). The capacity of dynein 1 to carry out all these different functions is surprising and it implies the presence of a tight regulation system to couple the right cargo and to exert the specific function.

Structurally, all forms of dynein consist of the same subunits: two heavy chains (HC), two intermediate chains (IC), two light intermediate chains (LIC) and a variable number of light chains (LC). The motor activity relies on the heavy chain polypeptide that contains 6 AAA ATPase domains arranged into a ring in the C-terminus. ATP hydrolysis by AAA1 and AAA3 are needed for motility, while ATP hydrolysis by

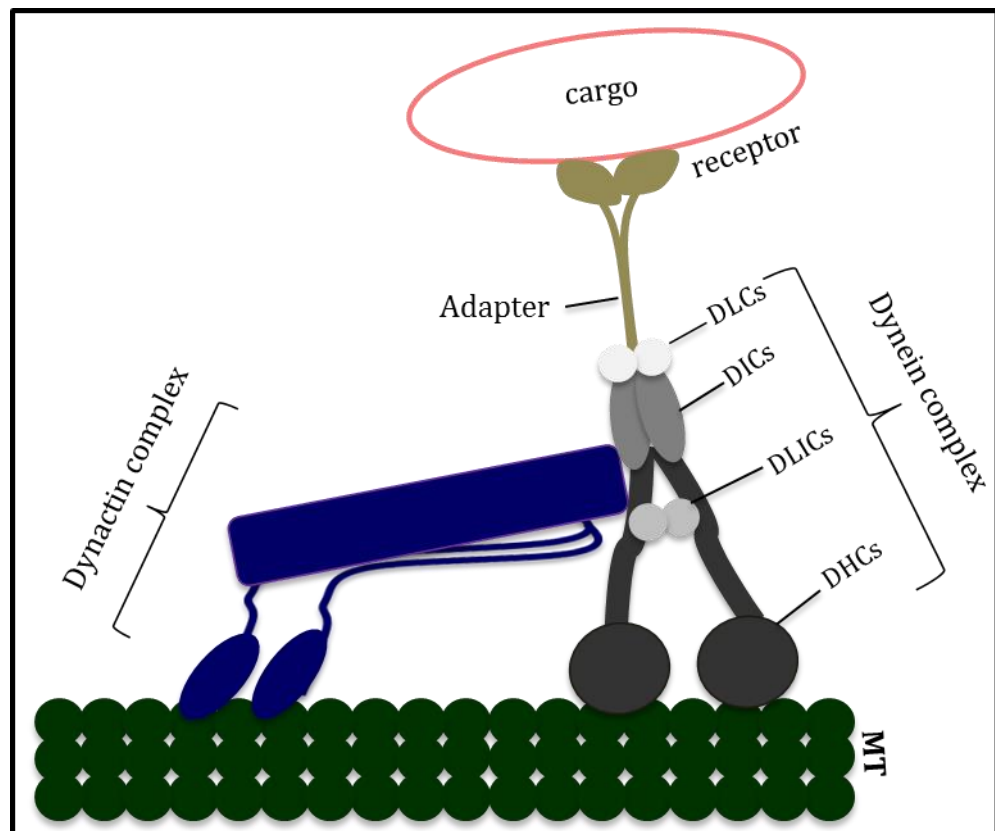
AAA2 and AAA4 seems to have regulatory role. Between AAA4 and AAA5 projects outwards the ‘stalk’, which contains the microtubule-binding site (Burgess *et al.*, 2003). The *N*-terminus domain of dynein mediates homo-dimerisation of the heavy chain and contains the cargo-binding domain (the ‘stem’). Within the ‘stem’ is the linker, a motile element that joins the tail and the AAA1 domain and changes position in response to nucleotide generating force (Burgess *et al.*, 2003). Moreover, the stem interacts with dimers of the accessory non-catalytic subunits that are also important for cargo binding.

The ICs are the largest accessory subunits and mediate interactions with different dynein adaptor proteins, such as the p150<sup>Glued</sup> (referred to herein as p150) subunit of dynactin and the ZW10 subunit of the RZZ complex allowing association with kinetochores (Vaughan and Vallee, 1995) (Whyte *et al.*, 2008). The LIC1 subunit, instead, interacts with pericentrin, facilitating dynein binding with centrosomes (Tynan *et al.*, 2000). Lastly, there are three classes of LCs that mediate binding with transmembrane receptors, ion channels and viruses (Allan, 2011)).

#### **1.4.1.1 Dynein Processivity**

Motor processivity is defined by the number of steps the motor can take before detaching from the microtubule. Yeast cytoplasmic dynein has been described as processive on its own, with a run length of 1/2  $\mu\text{m}$ , whereas mammalian cytoplasmic dynein can produce movements only when attached to a solid surface (Reck-Peterson *et al.*, 2006) (Toba *et al.*, 2006). Over the years it was discovered that different species of dynein can travel at different velocities: yeast dynein is much slower than *Dyctyostelium* dynein and metazoan dynein and they show irregular stepping behaviour (Reck-Peterson *et al.*, 2006) (Nishiura *et al.*, 2004) (Trokter *et al.*, 2012). It is possible to record not only 8 nm forward steps (the typical step size of kinesins), but also backward and lateral stepping (Reck-Peterson *et al.*, 2006) with mammalian dynein

showing even higher variability in the stepping size (from 8 to 32 nm) (Toba *et al.*, 2006). Dynein processivity requires not only intact linkers and dimerisation (Reck-Peterson *et al.*, 2006) but also purified dynactin, which has been shown to double the processivity of the motor (King *et al.*, 2003) (Culver-Hanlon *et al.*, 2006). Although, dynactin is a crucial activator of motor processivity, it has a weak affinity for dynein, suggesting the presence of another layer of regulation (King *et al.*, 2003) (McKenney *et al.*, 2014). It has been shown that dynein can engage different partners to modulate its functions, in terms of both processivity and cargo-specificity (Kardon and Vale, 2009) (McKenney *et al.*, 2014) (Fig. 1.7).



**Figure 1. 7. Cartoon of the tripartite-dynein motor complex.**

Dynein is not processive on its own; it requires interaction with adapter molecules (such as BicD2 or Spindly) and the dynactin complex. The adaptors mediate the link with the cargo, while dynactin enhance the microtubule binding. DHC: dynein heavy chain; DLIC: dynein light intermediate chain; DIC: dynein intermediate chain; DLC: dynein light chain.

#### ***1.4.1.2 Dynein regulatory factors***

The way in which dynein interacts with the innumerable cargos that it can transport towards the microtubule minus-end has not been properly elucidated yet. Only with complex regulation of processivity and targeting can dynein move the many specific cargos and execute all of the functions for which it is responsible. One hypothesis is that the specificity is defined by expressing splice variants of the different subunits of dynein; expression of different isoforms of ICs or LICs mediates association with different proteins (Kuta *et al.*, 2010). Additionally, binding different dynein adaptors allows loading of diverse cargos and execution of different functions (Kardon and Vale, 2009). The key adaptors of dynein are discussed in more details in the next paragraphs.

#### **Dynactin**

Dynactin is itself a multi-subunit complex shown to be involved in most, if not all, dynein activities (Schroer, 2004). It was reported as crucial for enhancing the processivity of the motor via its p150 subunit that interacts with the IC of dynein (King and Schroer, 2000) (Echeverri *et al.*, 1996). The p150 subunit binds to microtubules via the CAP-Gly (cytoskeleton-associated protein glycine rich) domain (Culver-Hanlon *et al.*, 2006), and it also associates with microtubule plus-end proteins, like EB1 (ending binding 1) and CLIP170 (CAP-Gly domain-containing linker protein 170) (Duellberg *et al.*, 2014). The microtubule plus-end binding seems to be related with the cargo loading and initiation of transport (Vaughan *et al.*, 2002). However, alterations in the microtubule binding site of dynactin do not affect the processivity of the motor (Kim *et al.*, 2007). To date, many studies have shown that the association between dynein and dynactin is not sufficient to activate motor processivity and cargo transport (Schlager *et al.*, 2014) (Zhang *et al.*, 2011). BicD, Spindly, Rab11-FIP3 and Hook3 have been

identified as important additional players in the activation of the processivity in the presence of the dynein/dynactin complex (McKenney *et al.*, 2014).

The p150 subunit links dynein to GTPases that mediate the trafficking from the endoplasmic reticulum (ER) to the Golgi apparatus: it interacts with the Rab-interacting lysosomal protein (RILP), a RAB7 GTPase associated with late endosomes (Johansson *et al.*, 2007). Other subunits of dynactin are likewise involved in the cargo-linking process: the p50/dynamitin subunit associates with Bicaudal D for intracellular trafficking and the ARP1 subunit associates with  $\beta$ III spectrin, a protein found on cytosolic surface of the Golgi apparatus and on other cellular membranes (Hoogenraad *et al.*, 2001) (Holleran *et al.*, 2001).

### Bicaudal D

Bicaudal D (BicD in flies or Bicaudal D1/D2 in mammals (BicD1/2)) has been widely described for its role for dynein-mediated transport, specifically for mRNA localisation in flies and for Golgi-vesicle transport in mammalian cells (Swan and Suter, 1996) (Hoogenraad *et al.*, 2001). Moreover, BicD functions in nuclear positioning via interaction with the nucleoporin RanBP2 in mammals, in lipid droplet transport and microtubule organisation in association with dynein (Splinter *et al.*, 2010) (Larsen *et al.*, 2008) (Fumoto *et al.*, 2006).

The interaction with the dynein/dynactin motor complex occurs at the *N*-terminus of the coiled coil of BicD and it dramatically increases the processivity of the motor (Urnavicius *et al.*, 2015) (Schlager *et al.*, 2014) (McKenney *et al.*, 2014); the *C*-terminus coiled coil instead mediates the interaction with specific cargos (Hoogenraad *et al.*, 2003). Interestingly it has been demonstrated that overexpression of BICD2-N (BICD2 *N*-terminus) prevents both the dynein cargo-binding and the MT association, suggesting that interaction with cargos is needed to induce a conformational change that

activates the triple complex (BICD2N-dynein-dynactin) and allows for MT binding to occur (Splinter *et al.*, 2012).

### Lis1

Lis1 was identified as the target of sporadic mutations causing human type I lissencephaly, a brain developmental disease resulting from defects in neuronal division and migration (Reiner *et al.*, 1993). Subsequently, Lis1 has been shown to localise in non-neuronal cells as a regulator of cytoplasmic dynein, being required for several dynein-dependent processes, such as mitotic spindle orientation, kinetochore activity, centrosome positioning, and mRNA and organelle transport (Smith *et al.*, 2000) (Faulkner *et al.*, 2000) (Kardon and Vale, 2009). Lis1 interferes with the coupling between ATPase activity and microtubule binding of dynein (Huang *et al.*, 2012). When Lis1 is bound to dynein, the motor stays attached to microtubules even upon several ATP hydrolysis cycles (Huang *et al.*, 2012). Alterations of Lis1 expression lead to massive defects in the functions of dynein at KTs: it delays anaphase onset and interferes with chromosome congression to the metaphase plate. Moreover, overexpression of Lis1 displaces dynactin from MT plus-ends in interphase cells (Faulkner *et al.*, 2000).

Lis1 can work on its own or in close collaboration with NudE (or NudEL), which promotes the tight binding of Lis1 to dynein mediating the force generation process (Efimov and Morris, 2000) (McKenney *et al.*, 2010). The Lis1/NudE/NudEL complex is required to target dynein to the microtubule plus-ends and is involved in many dynein-mediated activities such as cell migration, targeting of dynein onto cortical microtubules and correct functioning of the mitotic spindle (Dujardin *et al.*, 2003) (Lee *et al.*, 2003) (Faulkner *et al.*, 2000).

It is still unclear whether Lis1 associates with dynein on moving cargo *in vivo*.



### NudE/NudEL

Besides their role in association with Lis1, NudE and NudEL are also needed for several other activities of dynein on their own associating with the motor via three binding sites (Liang *et al.*, 2004) (McKenney *et al.*, 2011). NudE/NudEL localise at centrosomes and at kinetochores (Feng *et al.*, 2000) (Stehman *et al.*, 2007), where they arrive earlier than dynein, Lis1 and dynactin, and their inhibition prevents dynein localisation and arrests cells in metaphase (Stehman *et al.*, 2007). NudE does not interact with dynactin, suggesting that NudE/NudEL play a primary role in kinetochore assembly and microtubule attachment functions of dynein, while the dynein pool that interacts with dynactin appears to be mainly responsible for mediating the SAC stripping function (Stehman *et al.*, 2007).

Outside mitosis, NudE and NudEL are further implicated in mediating dynein binding to cellular membranes for organelle positioning; depletion of either NudE or NudEL generates loss of dynein from those membranes (Lam *et al.*, 2010).

### The RZZ complex

The RZZ complex is made up of three different proteins: Rod, Zwilch and ZW10 (Karess, 2005). It has been reported as a main player in the recruitment of the dynein/dynactin complex to kinetochores (Starr *et al.*, 1998) (Whyte *et al.*, 2008); it can physically link dynein to KTs and induce dynein-mediated kinetochore-movements (Varma *et al.*, 2013). ZW10 (or Zeste white 10) binds to the motor complex directly, upon phosphorylation of the IC of dynein, and indirectly, through interaction with the p50 (/dynactin) subunit of dynactin (Starr *et al.*, 1998) (Whyte *et al.*, 2008). The RZZ complex is important for two kinetochore functions mediated by dynein: chromosomes alignment and silencing of the SAC signalling. Both functions rely upon the recruitment of Spindly, a binding partner of the complex in mitosis. By promoting the association of

the dynein/dynactin motor complex to KTs, the RZZ complex induces its own removal, together with the SAC proteins, stimulating the inactivation of the checkpoint (Howell *et al.*, 2001) (Karess, 2005). This process of removal allows also the stabilisation of stable ‘end-on’ attachments since it increases the binding affinity of Ndc80 for MTs by relieving the inhibition due to the presence of RZZ at KT (Barisic and Geley, 2011). However, RZZ and dynein/dynactin have different dwell times at KTs; RZZ exchanges on and off of kinetochores much slower than the motor (Famulski *et al.*, 2008).

Besides the role in mitosis, ZW10 plays an alternative role in a dynein-mediated process in interphase. It localises to the Golgi apparatus and it promotes dynein-association with it inducing minus-end-directed movements of the Golgi itself, endosomes and lysosomes (Hirose *et al.*, 2004) (Varma *et al.*, 2006) (Civril *et al.*, 2010).

### Spindly

Spindly has been widely described as a dynein co-factor at kinetochores during mitosis (Griffis *et al.*, 2007) (Gassmann *et al.*, 2008) (Chan *et al.*, 2009; Cheerambathur *et al.*, 2013; Holland *et al.*, 2015). It has been reviewed earlier in this chapter (see paragraph 1.3), so it will not be analysed again in this paragraph.

## 1.5 Mitotic proteins in interphase

Over the years researchers have widely demonstrated that many proteins are able to play alternative roles in several pathways different from those where they were initially identified. It is believed that proteins have evolved to carry out second/additional function(s) that can differ according to cell cycle state, cellular localisation, cell type, oligomeric state or different proportion of a ligand/substrate ratio. There could be several inputs that work together to switch a protein function. Jeffery denominated those proteins with multiple roles as ‘moonlighting protein’ in 1999 (Jeffery, 1999).

To date have been identified several mitotic proteins that are able to play additional roles in interphase.

Dynein is an important “moonlighting protein”. It can mediate a wide range of functions, including virus transport, mitotic division, cell migration, lysosome and late endosomes transport and nuclear migration (Vallee *et al.*, 2012). Dynein can interact with many regulatory factors (some of them already described) that seem to facilitate its capacity to play several functions and to load different cargos according to the phase of the cycle the cell is in and to the cellular compartment where dynein is expressed (Kardon and Vale, 2009). As already stated there are numerous dynein interactors that could mediate the linking to cargos suggesting the presence of potential diverse cargo recruitment mechanisms (Akhmanova and Hammer, 2010). This fine regulation of dynein depicts it as maybe the best “moonlighting protein” identified to date. In this context, different dynein adaptors have been proved to behave as “multifunctional” proteins on their selves. For instance, Lis1 plays a role in brain development (Reiner *et al.*, 1993) as well as in cell division, cell migration and nuclear migration (Faulkner *et al.*, 2000) (Smith *et al.*, 2000) (Dujardin *et al.*, 2003)

NudEL, a close binding partner of Lis1 and dynein implied in regulation of some dynein/Lis1-mediated functions, is also described playing a role in organelles

trafficking. It interacts with ER/Golgi membrane, synaptosomal membrane and synaptic vesicle and it possesses a Lis1-binding site and a dynein-binding site that have to be intact to allow proper functioning of the protein and correct mediation of the motor activity. Mutations on one or both of these binding sites generate fragmentations/dispersions of the Golgi cisternae, lysosomes and endosomes (Liang *et al.*, 2004).

Furthermore, ZW10, a component of the RZZ complex originally identified as a *D. mel.* mutant involved in kinetochore function (Chan *et al.*, 2000), is involved in membrane trafficking between the ER and the Golgi apparatus, being associated with ER membranes via an interphase specific interaction with RINT-1 (Hirose *et al.*, 2004). These proteins, associated with a third one called NAG, make up the NRZ complex (NAG/RINT-1/ZW10) that is associated to the ER through the ER SNAP-receptors syntaxin 18 and BNIP1 (Civril *et al.*, 2010). ZW10 was shown also interacting with the Golgi apparatus as a consequence of its movements to the minus-end of microtubules, in association with dynactin through which it could be assisting dynein-mediated endocytosis (Starr *et al.*, 1998) (Varma *et al.*, 2006). Furthermore, ZW10 was recorded to localise at the leading edge of migrating cells potentially involved, also in this case, in the recruitment of dynein at the cortex of lamellipodia (Varma *et al.*, 2006).

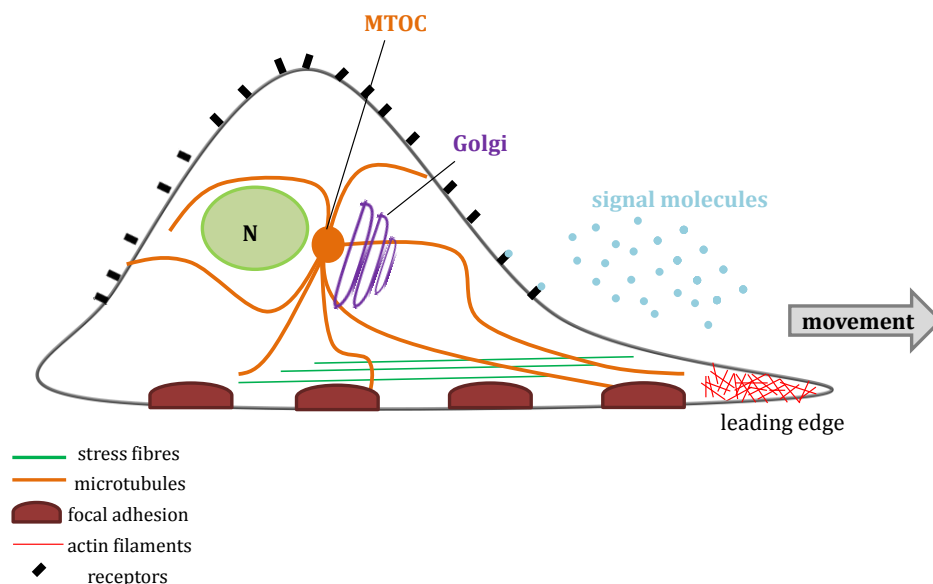
Zwint, an important kinetochore protein in mitosis, has been described as a player in membrane trafficking as well via its interaction with Rab3c (van Vlijmen *et al.*, 2008).

Mad1, an important SAC molecular player, possesses also an alternative role in interphase, binding to the Golgi apparatus and co-localising with Rab6A in both *trans*-Golgi network and endosomes (Wan *et al.*, 2014). Moreover, Mad1 depletion was

shown to decrease the migratory capacity of cells: indeed, the presence of Mad1 at the Golgi apparatus promotes  $\alpha 5$ -integrin secretion that in turn stimulates cellular adhesion, spreading and motility, which ties the interphase localisation and functioning of Mad1 to cell migration (Wan *et al.*, 2014). For this interphase function Mad1 does not require Mad2 interaction; conversely, for its interphase localisation at the nuclear pore complex, it strictly depends on Mad2 presence, even though their function in that context has not been elucidated yet (Campbell *et al.*, 2001).

## 1.6 Cell migration

An important process regulated by the cytoskeleton network in cells is represented by migration, a vital mechanism in many biological contexts, in both normal physiology as well as in pathological conditions. It is central for tissue development during embryogenesis, but also for wound healing, tumour metastasis and inflammatory responses (Lauffenburger and Horwitz, 1996). The activation of this process requires specific signals, represented by growth factors, chemokines or extracellular matrix molecules, which activate specific receptors and signalling cascades (Fig. 1.8) (Ridley *et al.*, 2003).



**Figure 1. 8. Schematic representation of a migrating cell.**

Signal molecules bind to receptors and promote cell polarisation with subsequent activation of Rho GTPases and formation of protrusions (leading edge) towards the direction of migration and adhesions with ECM and surrounding cells. N: nucleus.

### 1.6.1 Cell polarisation and protrusions formation

To migrate, cells must polarise defining a protruding front (the side closest to the direction of migration) and a retracting rear (opposite side to the front) to promote translocation of the cell body. Polarisation is often dictated by the extracellular

environment and it requires modifications in the actin cytoskeleton distribution and asymmetric activation of cell-membrane receptors. The assembly and polymerisation of actin at the cell front stimulates the extension of flat membrane protrusions to initiate the migration cycle. Actin dynamically polymerises to generate filaments that represent the basal structure for cell movement; polymerisation occurs at the plus end and is usually correlated with the disassembly at the minus end (Alberts *et al.*, 2007). The process is divided in three phases: nucleation (three actin monomers generally interact), elongation (monomers are rapidly added to the filament at the plus (or barbed) end) and steady state (monomers disassembly from the minus end and at the same time polymerisation is maintained at the plus end by monomers present in the cytosol) (Alberts *et al.*, 2007).

There are different types of protrusion according to the organisation of actin filaments (Yamaguchi and Condeelis, 2007) (Krause and Gautreau, 2014) (Ridley, 2015):

- Lamellipodia: broad, flat protrusions at the leading edge that contain a branched network of actin filaments and elongate when cells migrate along the extracellular matrix; these protrusions require Rac GTPase activation for actin polymerisation and Integrin-mediated adhesion that also maintains Rac GTPase activity in a positive feedback loop. Lamellipodia are transient structures that protrude and retract.
- Filopodia: thin finger-like protrusions that extend from the cellular membrane and are made of long, unbranched actin bundles. Filopodia can support residual slow migration in the absence of lamellipodia and they consent the cell to probe the surrounding environment. Cdc42 is the best GTPase involved in filopodia formation, acting through formins to stimulate actin polymerisation (Kuhn and Geyer, 2014).
- Invadopodia: often generated in cancer cell migration and specialised to invade through the basement membrane by releasing several molecules and most importantly matrix

metalloproteases that allow for degradation of the ECM. Rho GTPases are well studied to play a role in invadopodia formation: Cdc42 has been reported to act through N-WASP for this process as well as other Cdc42 GEFs proteins (Beaty and Condeelis, 2014).

- Podosomes: conical, actin rich structures localised at the substrate-attached part of the cell that present an F-actin-rich core surrounded by a ring structure characterised by adhesion and scaffolding protein. These protrusions are highly dynamic and actively engaged in matrix remodelling and tissue invasion.
- Blebs: outward bulges in the plasma membrane driven by hydrostatic pressure from inside the cell. In this case the actin-Myosin cytoskeleton is involved: while Myosin 2 generates contraction, providing the contractility necessary for the extension of the blebs, actin assembly provides them stability. To activate this type of migration cells need to upregulate the RhoA/ROCK pathway (Sanz-Moreno *et al.*, 2008).

Several proteins regulate the rate and organisation of actin polymerisation in protrusions by affecting the pool of available monomers and free ends. The Arp2/3 complex, for example, is expressed in lamellipodia where it binds to the side of an existing actin filament promoting the branching of a new filament by stimulating actin nucleation via activation of the Wiskott-Aldrich syndrome family proteins (WASP, N-WASP, WAVE and WASH proteins) (Skoble *et al.*, 2000). This family of proteins mediate the interaction between Arp2/3 and the actin so to constitute the nucleation core (Pollard, 2007). Many other actin-nucleating proteins are involved in this process, regulating the rate and the organisation of actin polymerisation and orchestrating the availability of actin monomers and free ends and controlling the architecture of the leading edge (dos Remedios *et al.*, 2003). Spire for instance binds to the rear-facing pointed ends of filaments and prevents depolymerisation of actin (Quinlan *et al.*, 2005);



Formins instead, bound to the rapidly growing barbed end of nascent actin filaments, mediate nucleation of linear unbranched actin filaments, leading to the formation of actin cables, filopodia or stress fibres (Goode and Eck, 2007).

### 1.6.2 Focal adhesions

Protrusions are stabilised by interactions with adjacent cells or with the extracellular matrix (ECM) (Le Clainche and Carlier, 2008). Interactions with the ECM, a complex network of polysaccharides (such as glycosaminoglycan or cellulose) and proteins (such as collagen and fibronectin) secreted by cells, are mediated by Integrins, migration-promoting receptors that recognise particular motifs in the ECM component fibronectin and support both adhesion and intracellular signalling. Integrins form clusters, or ‘focal complexes’, which recruit focal adhesion kinase (FAK), Rho and Myosin 2 to allow assembly of actin and generation of traction forces on filaments (by mediating association with structural proteins such as Vinculin,  $\alpha$ -actinin, Paxillin and Talin (Kanchanawong *et al.*, 2010)) and at the same time the maturation of a ‘focal complex’ into a ‘focal adhesion’ (Miyamoto *et al.*, 1995).

Movements rely on traction forces generation upon connection between actin stress fibres and the ECM. Focal adhesion sites in this context act as molecular clutch that tethers to the ECM and impedes the retrograde movement of actin, allowing for generation of traction forces at the site of adhesion. However proper movement requires not only constant formation of focal adhesion sites but also their disassembly at the rear, to promote retraction of the cell tail and detachment to allow forward movement of the cell body (Crowley and Horwitz, 1995).

Adhesions close to the leading edge normally are highly dynamic and rapidly form and turn over (Webb *et al.*, 2002). This process is mediated by reorganisation of actin filaments and recruitment of several molecules, such as FAK, Src and calpain; also

microtubules play a role by modulating their contraction forces (Webb *et al.*, 2002) (Ren *et al.*, 2000). The probability that a nascent adhesion in the lamellipodium matures, rather than disassembles, seems to depend on the level of active Myosin 2, which is involved in their elongation and growth by mediation of actin bundling process (Choi *et al.*, 2008). The role of Myosin 2 as a regulator of protrusion is related with its capacity to generate forces on adhesions without interacting with them but by attaching to the actin bundles with which adhesions are associated (Vicente-Manzanares *et al.*, 2007). Indeed, increased levels of active Myosin 2 results in large actin bundles and stable adhesions, leading to decrease of signalling to Rac GTPase at the front of the cell and protrusion formation. On the other side, low levels of active Myosin 2 lead to less actin bundling and increased protrusion formation (Vicente-Manzanares *et al.*, 2009). The recruitment of Myosin 2 is itself related with the stretch of actin filaments; at the rear of the cell, actin filaments interact with the intracellular portion of focal adhesions resulting in being more stretched compared with those at the front of the cell that instead interact with the plasma membrane. This results in higher levels of Myosin 2 associated with filaments at the rear of the migrating cell, generating an unequal distribution of tension (Chi *et al.*, 2014). Myosin 2 is activated upon phosphorylation of the regulatory light chain (RLC) that increases its activity in the presence of actin. Phosphorylation is mainly dependent on two kinases: the Rho kinase (ROCK), activated by Rho GTPase, and the Myosin light chain kinase (MLCK) that regulates the activity of Myosin 2 and actin, driving cell contractility (Etienne-Manneville and Hall, 2002) (Riento and Ridley, 2003). Rho/ROCK signalling mediates the retraction of the trailing edge of cells and it has been implicated in adhesion disassembly during cell detachment; indeed inhibition of ROCK or MLCK induces an elongated morphology with impaired rear-end detachment (Worthylake *et al.*, 2001). Eventually, this process is inactivated by the Myosin phosphatase that mediates the de-phosphorylation process.

### 1.6.3 Rho GTPases

Key regulators of cell shape changes and protrusion formation are proteins from the Rho family of small guanosine triphosphate (GTP)-binding proteins (GTPases), like Rac, Rho and Cdc42. Such proteins are active only when bound to GTP and in this conformation they can activate the downstream pathways. In their inactive state, so when they are bound to GDP, Rho GTPases are free in the cytoplasm and associate to the guanine nucleotide displacement inhibitor (GDI); growth factors can then bind and activate their receptors to turn on specific membrane-bound regulatory proteins called guanine nucleotide exchange factors (GEFs) to activate Rho proteins at the membrane by releasing them from GDI and promoting the exchange of GDP for GTP (Ridley, 2015). The GTP-bound Rho protein can in turn associate with the plasma membrane and bind downstream effector proteins to initiate the biological response. Therefore, according to the amount of small G proteins active the expression of Rho GTPases can be locally regulated in different regions of the cell.

Rac is generally active at the front of the cell where it stimulates formation of protrusions regulating actin polymerisation and branching of actin filaments by inducing the activation of Arp2/3 complex by WAVE family proteins. Rac also inhibits the disassembly of actin filaments by inactivation of actin depolymerising factors, such as ADF/Cofilin (Hall, 1998).

Rho is required to regulate the contraction and retraction forces in the cell body and at the rear; at these regions it is also needed for maturation of existing contacts (Raftopoulou and Hall, 2004). Rho is involved in the bundling process that involve actin and Myosin 2 in order to generate contractile actin-myosin bundles, like stress fibres, and to make up new focal adhesion sites through the activation of the Formin Diaphanous (Dia) and ROCK.

Rho and Rac also interplay with each other by antagonising each other to maintain cells polarity by preventing the formation of Rac-mediated protrusion in sites that are not the front of the cell (Raftopoulou and Hall, 2004).

Important in cell migration is also the Rho GTPase Cdc42, active at the front of the cell, that, together with dynein, mediates the re-localisation of the MTOC and of the Golgi apparatus in front of the nucleus towards the leading edge to polarise cell migration and guide vesicle transport towards the leading edge (Palazzo *et al.*, 2001) (Magdalena *et al.*, 2003). Additionally, Cdc42 is required for polymerisation of F-actin during protrusion formation by activation of the Arp2/3 complex through WASP family proteins (Hall, 1998) (Etienne-Manneville and Hall, 2002).

#### 1.6.4 Microtubules in cell migration

Microtubules are also involved in establishing polarity and re-orienting the MTOC and therefore are essential for cell migration (Watanabe *et al.*, 2005).

Depolymerisation of microtubules promotes formation of focal adhesions whereas their polymerisation induces lamellipodia formation. The assembly state of microtubules is directly related with cell migration since it can modulate the activity of Rho GTPases: at the leading edge of fibroblasts the concentration of RhoA is increased upon MT depolymerisation (Ren *et al.*, 1999), while instead MT polymerisation activates Rac1 (Waterman-Storer *et al.*, 1999). Many microtubule associating proteins (MAPs) are implicated in microtubule stabilisation at the leading edge, tethering microtubules to actin filaments and crosslinking these two cytoskeleton structures (Rodriguez *et al.*, 2003). For instance, CLIP170 plays a role in cell polarity by associating with IQGAP, an actin-binding protein that binds on one side with Rac and Cdc42 and on the other side with CLIP170 behaving as a cross-linker (Fukata *et al.*, 2002). MACF 1/Spectraplakins also mediate this cross-interaction since it co-localises

with and binds to microtubules and actin cytoskeleton (Leung *et al.*, 1999). Cytoplasmic dynein and dynactin seem to play a similar function; they co-localise with F-actin at the leading edge of fibroblasts and participate in MTOC re-distribution for cell polarisation; defects in dynein expression interferes with directed cell movements and with centrosome re-organisation (Etienne-Manneville, 2001) (Palazzo *et al.*, 2001) (Dujardin *et al.*, 2003).

Actin filaments and microtubules can associate to one another *in vivo* affecting their organisation and dynamics. A retrograde flux of actin filaments (toward the cell centre) generates a backward force on microtubules that actively pushes them away from the direction of migration (Salmon *et al.*, 2002). Dynein and other cross-linkers can either resist this retrograde flow to link the actin network and MTs to the leading edge or they can act as a membrane anchor for MTs to allow the invasion of the actin network at the front of the cell (Salmon *et al.*, 2002).

Microtubules interact with focal adhesions as well promoting their turnover (Kaverina *et al.*, 1998).

Hence, microtubules influence cell motility by controlling actin assembly, adhesion turnover and cell polarity; many different factors are involved in this crosstalk that is critical to allow communication and interplay between these two cytoskeleton networks generating also cooperatives effects and feedback loops that stimulate both sides (Etienne-Manneville, 2013).

### 1.6.5 Vesicle transport

As stated earlier, cell migration requires polarisation of the cell, a process that allows identifying a leading edge and a trailing end. For this, a plethora of proteins have to be spatially segregated and confined within the cell. The intracellular localisation of proteins that are membrane associated - either due to a membrane anchor or via binding of a membrane-associated protein - depends predominantly on vesicular trafficking.

Therefore cells possess an active ‘polarised’ endo-exocytic process that mediates the targeting of such proteins, for instance Integrins, to specific regions of the cell surface (Maritzen *et al.*, 2015). Many studies indicate an increase in the rate of internalisation of endocytosis at the leading edge of migrating cells; receptors are internalised into early endosomes and then either targeted for degradation or recycled to the plasma membrane (Jones *et al.*, 2006). The major route for internalisation is through clathrin-mediated endocytosis, which is initiated upon chemokine binding to the receptor and its subsequent phosphorylation. Once internalised, these receptors can either proceed to late endosomes/lysosomes to be degraded or can dissociate from the ligand and enter the recycling compartment to traffic back to the plasma membrane.

Small GTPases of the Rab family are responsible for the regulation of the intracellular trafficking steps; importantly they undergo post-translational modification by isoprenylation, generating hydrophobic moieties that facilitate membrane association. Rabs are inactive when bound to GDP and in this state are distributed to the cytosol complexing with GDI that interacts with the isoprenylated C-terminus of the Rabs and blocks their dissociation from GDP. Subsequently, once on appropriate membrane, the GEFs promote the exchange of GDP to GTP activating the Rab that in turn recruits downstream ‘effector’ proteins, such as tethering factors, kinases, phosphatases, scaffold proteins and actin/microtubule-based motor proteins (Somsel Rodman and Wandinger-Ness, 2000). An example of a crucial endocytic trafficking for cell migration is represented by the internalisation of Integrins. This process is triggered by the removal of active, GTP-bound, Rac from the membrane, which leads to turning off of Rac and of the protrusive activity of cells (del Pozo *et al.*, 2004). The Integrin-trafficking can occur via two potential pathways: one under the control of Rab4 GTPase (called ‘short-loop’) and one under the control of Rab11 GTPase (called ‘long-loop’) (Maritzen *et al.*, 2015). The ‘long loop’ is characterised by endosomes that pass through

the perinuclear recycling compartment before reaching the plasma membrane and it is mediated as well by the ARF subfamily of GTPases that also control the actin cytoskeleton, integrating vesicular transport with actin polymerisation (Jones *et al.*, 2006). As well as Integrins, also other cell surface adhesions proteins are recycled from the plasma membrane by endocytosis regulating the cell migration process (like syndecans or E-cadherin) (Jacquemet *et al.*, 2013).

An interesting feature of Rabs is their capacity to regulate microtubules motors and their capacity to attach membranous cargo to the microtubule cytoskeleton. For example, Rab14 was demonstrated to interact with KIF16B (kinesin-3 subunit) regulating in this way Golgi-to-endosome trafficking of the FGFR (fibroblast growth factor receptor). Although Rabs can associate directly with kinesin motor proteins, often they can bind to adaptor proteins first and only subsequently to different kinesin complexes. Moreover, some Rabs can interact with multiple kinesins and regulate distinct trafficking processes: for example Rab6 associates with kinesin-1 and 3 via BICD and BICDR-1 (BICD-related protein 1) respectively regulating trafficking processes (Grigoriev *et al.*, 2007).

On the other side, Rab GTPases can interact with the minus end microtubule-based motor protein dynein as well, directing a wide range of intracellular trafficking steps. Rab7, for instance, associates with dynein through the binding of RILP (Rab interacting lysosomal protein) to dynactin (p150) to control endosomal transport (Jordens *et al.*, 2001) Rab11A interacts with dynein light chain subunits controlling the endosomes sorting process (Horgan *et al.*, 2010). Also Rab6, already shown to interact with kinesin, can associate with dynein as well via binding dynactin (p150 and p50 subunits) and regulating in this way the trafficking steps in the biosynthetic pathway (Hoogenraad *et al.*, 2001).

## 1.7 Aims and objectives

Since its discovery Spindly has been described as an important mitotic player, in close relation with the dynein/dynactin motor complex and with the spindle assembly checkpoint (SAC). Previous studies have clearly shown that the silencing of the SAC requires the recruitment of the dynein/dynactin motor complex onto kinetochores and that this loading is highly dependent on Spindly presence (Griffis *et al.*, 2007) (Gassmann *et al.*, 2008) (Chan *et al.*, 2009).

Even though this model is widely accepted, direct interaction between Spindly and dynein/dynactin has been inadequately studied and the function of Spindly in the SAC has not been fully addressed. Paradoxically it has been demonstrated that Spindly-depleted cells can somehow remove SAC components from kinetochores without recruiting dynein/dynactin, while the presence of Spindly mutants on attached kinetochores prevents SAC silencing, suggesting a much more direct role for Spindly in SAC activation/maintenance (Gassmann *et al.*, 2010).

This thesis aims to closely dissect the interaction between Spindly and the dynein/dynactin motor complex and to understand the contribution of Spindly within the spindle assembly checkpoint pathway.

Additionally, to date, little is known regarding human Spindly outside mitosis either on its own or in relation with other proteins/cellular activities. Previously it was shown that depletion of *Drosophila* Spindly can generate defects in cytoskeletal organisation (Griffis *et al.*, 2007). Therefore, this work seeks to unravel the role of Spindly in interphase, with a close look at its function in relation to cytoskeleton (and cell migration).

It is true that many proteins have been shown to play two or more additional functions within a cell beside the one for which they were initially described; so with this thesis we plan to understand whether this is the case for Spindly, believing that it



could represent a versatile scaffold protein able to bring together multiple complexes in different biochemical processes.

## **2 . Material and methods**

## 2.1 Cell lines and growth conditions

Human embryonic kidney cells HEK293 (kindly gifted by Prof. D. R. Alessi, University of Dundee), human osteosarcoma U2OS cells (kindly gifted by Dr. S. Rocha, University of Dundee) human cervix carcinoma cells HeLa (ATCC), human keratinocyte cells HaCaT (kindly gifted by Dr. L. Unterholzner, University of Dundee), mouse embryonic fibroblast (MEF) cells (kindly gifted by Dr. V. Cowling, University of Dundee) and human foreskin fibroblast (FB) cells (kindly gifted by prof. A. Huebner, Dresden) were maintained in Opti-MEM (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS) (Gibco), 1% Penicillin, Streptomycin and Glutamine (Gibco) for no more than 30 passages.

Cell lines were grown at 37°C with 5% CO<sub>2</sub> in a humidified incubator.

When the experiment required mitotic enrichment, cells were treated with the indicated drug for the specified period of time before being collected

## 2.2 Generation of stable cell lines

U2OS cells with an integrated FRT site and a Tet Repressor (kindly gifted by Prof. A. Lamond, University of Dundee), were kept in medium with Zeocin (100µg/mL; Invitrogen) and Blasticidin (15µg/mL; Calbiochem). 48 hours prior to transfection, Zeocin and Blasticidin were removed from the culture medium. The pOG44 vector (a kind gift from Prof. J. R. Swedlow, University of Dundee) and the wild type or the mutant (F258A and S256A) version of the pCDNA5/FRT/TO LAP-Spindly constructs (Gassmann *et al.*, 2010) were co-transfected in a ratio of 9:1 pOG44: pgLAP vector into U2OS cells. After 48 hours from transfection stable integrating cell lines were drug selected by using 150 µg/mL of Hygromycin (Calbiochem) and 15 µg/mL of Blasticidin, and clonally isolated (see **Chapter 7**. Appendix- Fig. 7.1).

Similarly, human cervical carcinoma cells (HeLa) with the same integrated FRT and TetRepressor system as well as an expression vector containing the GFP-Spindly constructs (either wild type or mutants) were generated in Dr. A. Saurin's laboratory (University of Dundee) and donated to us.

All cells were grown in MEM (Gibco) supplemented with 10% HI-FBS, 1% Penicillin, Streptomycin and Glutamine (Gibco) for no more than 30 passages. Expression of the GFP construct was induced by administration of doxycycline (0.1-1  $\mu$ g/mL) (Calbiochem).

## **2.3 Small Interfering RNA (siRNA) Transfection**

Small interfering RNA oligonucleotides were synthesized by SIGMA and transfected into cells using Lipofectamine RNAiMax (Invitrogen) according to manufacturer's instructions. The oligonucleotide sequences used for siRNA knockdown are as follows: a GC-matched non-targeting control (MISSION Negative Control, SIGMA) diluted to a final concentration of 20nM; Spindly Endo1 (GAAAGGGUCUCAAACAGAA) and Spindly-UTR-66 (CUUGAUCUGACAUAUAUCA) (neither of which target the expressed Spindly constructs) combined together to a final concentration of 20nM. Cells were seeded and directly treated. Treatment was left on for 96 hours and then cells were fixed, harvested or seeded again for subsequent analysis.

## **2.4 Overexpression transfection**

DNA transfection procedure was carried out using FuGene HD (Promega) according to manufacturer's instructions. Cells were transfected 24 hours after being seeded with FuGene/DNA in a ratio of 3:1 incubated in 200 $\mu$ L of serum-free media. The mix was dropped onto cells growing in MEM supplemented with 10% FBS and plates were incubated at 37°C for at least 24 hours before experiment was conducted.

All the DNA plasmids used in this study were purified using the QIAprep Spin Miniprep Kit (QIAGEN), following manufacturer's instructions.

## **2.5 Protein Lysis**

Cells were lysed using the following lysis buffer: 50mM Tris/HCl pH 7.5, 150 mM NaCl, 100mM N-Ethylmaleimide (NEM), 0.3% CHAPS, 1mM EGTA, 1mM EDTA, 10mM Na- $\beta$ -glycerophosphate, 1mM Na-orthovanadate, 50mM Na-Fluoride, 10mM Na-pyrophosphate, 270mM sucrose, 0.1mM PMSF, 1mM Benzamidine, 0.1%  $\beta$ -mercaptoethanol, 1 protease inhibitor cocktail tablet (Roche) for 10 mL of buffer. Cells were then transferred into Eppendorf tubes and put under constant agitation for 10 minutes at 4°C. Samples were subsequently spun down for 15 minutes at 13,000 rpm at 4°C. Supernatants were collected and stored at -80°C.

## **2.6 Western blot**

Protein concentration was measured using Bradford dye (BioRad), accordingly to manufacturer's instructions. Samples were prepared in 2x loading buffer (LDS sample buffer, Novex-Life Technologies) and boiled at 95°C for 5 minutes. Soluble fractions were resolved on Tris-glycine SDS-PAGE gels (4-12% gradient gel; Novex NuPAGE SDS-PAGE Precast Gels, Life Technologies) run at 120-150 volts. Running buffer used was 50mM MOPS, 50mM Tris Base, 0.1% SDS, 1mM EDTA.

The gels were then transferred to nitrocellulose membrane (Amersham Protran 0.2 NC, GE Healthcare) for 2 hours at 100 volts in transfer buffer (25mM Tris Base, 192mM glycine, 0.1% SDS, 10% Methanol). The membrane was blocked in 5% milk dissolved in Tris-buffered saline [TBS]-0.2% Tween 20 for 1 hour and subsequently probed with primary antibodies overnight. Antibodies used are showed in **Table 2.1**.

Membranes were subsequently washed three times in TBS-Tween buffer and incubated with HRP-conjugated secondary antibodies (Cell Signalling) for 1 hour. Membranes were subsequently washed with TBS-Tween buffer and developed using ECL solution (Pierce) onto X ray film (Konica Minolta).

**Table 2. 1.List of antibodies used.**

ANTIBODY	MANUFACTURE	SPECIES	DILUTION
Spindly	(Griffis <i>et al.</i> , 2007)	Rabbit	1:5000
DIC	(Vaughan and Vallee, 1995)	Rabbit	1:1000
ZW10	AbCam	Rabbit	1:1000
Mad2	ThermoScientific	Rabbit	1:1000
Mad1	Bethyl Laboratories	Rabbit	1:1000
Bub1	Bethyl Laboratories	Rabbit	1:1000
BubR1	Bethyl Laboratories	Rabbit	1:1000
GFP	Novus Biological	Sheep	1:5000
p50	BD Biosciences	Mouse	1:1000
p150	SantaCruz	Mouse	1:1000
DHC	SantaCruz	Rabbit	1:1000
FLAG	Sigma	Mouse	1:2000
PCNA	SantaCruz	Mouse	1:200
Geminin	SantaCruz	Rabbit	1:200
pHistoneH3	Novus Biological	Rabbit	1:500
GAPDH	SantaCruz	Mouse	1:1000
CENP-E	SantaCruz	Goat	1:1000
CENP-F	SantaCruz	Goat	1:1000

## 2.7 Immunoprecipitation

200 µg of protein from whole cell lysate were incubated overnight at 4 °C under constant agitation with 1 µg of antibody in buffer D (20nM HEPES pH 7.9, 1mM DTT, 0.1% NP40, 20% glycerol, 1mM PMSF, 5mM NaF, 500 µM Na<sub>3</sub>VO<sub>4</sub> and 1 protease inhibitor cocktail tablet (Roche) per 10 mL of final volume).

Normal IgG was always used as negative control. Protein G Sepharose beads (Generon) or Protein A magnetic beads (Invitrogen) were added for 2 hours at 4°C under constant agitation. Precipitates were subsequently washed twice with lysis buffer and twice with PBS 1X. Washes were carried out under constant agitation at 4°C (10 minutes incubation for each wash). Loading buffer (2x) was then added to the beads that were boiled for 5 minutes and resolved by Western Blot analysis (see **2.6**).

## **2.8 Gel filtration**

### **2.8.1 Superose 6 column**

HEK293 cells were collected and lysed as previously described (see **2.5**). Cells were then transferred into Eppendorf tubes and put under constant agitation for 10 minutes at 4°C. Samples were subsequently spun down for 15 minutes at 13,000 rpm at 4°C and supernatants were collected. 200 µL of samples were then loaded into the Superose 6 column (10x300mm, 13µm particles, 40nm pores, GE Healthcare) equilibrated in lysis buffer. Prior to running our samples we calibrated the column by loading the standards (100 µL injection) diluted as follow: IgG (0.5mg/mL); BSA (1mg/mL); trypsin inhibitor (1mg/mL). Experiments were conducted at 4°C. Fractions were collected in a 96-well plate (Eppendorf, Deepwell plate 96/1000 µL) and resolved in 2x LDS sample buffer. Western Blot was then carried out to identify elution profile of our proteins of interest. Antibodies dilution as previously stated (see **Table 1**).

### **2.8.2 SEC-1000 column**

HEK293 cells were collected and lysed (see **2.5**), sonicated for 30 minutes (30'' pulse on/ 30'' pulse off) at 4°C and spun down for 10 minutes at 4°C at 13,000 rpm. Supernatant was collected and filtered using ultrafree-MC centrifugal filter units (pore size 0.45 microns, Millipore). Samples were then injected into the column (200 µL



injection). Column used was BioBasic SEC-1000 (7.8x300mm, 5µm particles, 1000Å pores, ThermoScientific) equilibrated in lysis buffer. Prior running our samples column was calibrated by loading the standards (100 µL injection), diluted as follow: IgG (0.5mg/mL); BSA (1mg/mL); trypsin inhibitor (1mg/mL). Experiments were conducted at room temperature.

Fractions were collected in a 96-well plate (Eppendorf, Deepwell plate 96/1000 µL) and resolved in 2x LDS sample buffer. Western Blot was then carried out to identify elution profile of our proteins of interest. Antibodies dilution as previously stated (see **Table 2.1**).

### 2.8.3 Double thymidine block

Cells were mitotically enriched by double thymidine block. First incubation with thymidine (2mM, Sigma) was carried on for 18 hours, and then cells were washed in PBS and released in complete media for 9 hours. Subsequently cells were incubated again with thymidine for 12 hours, washed with PBS and released in complete media for 6 hours. Nocodazole (40ng/mL, Calbiochem) was added for 16 hours and cells were finally harvested by mitotic shake-off, lysed and ran through the column.

## 2.9 Mass spectrometry analysis

HEK293 cells were seeded into 15cm petri dishes and once confluent treated overnight either with Nocodazole (40ng/mL) or S-trytil-L-cysteine (STLC, 5µM; Enzo, Life Sciences) to enrich the mitotic population. Asynchronous cells were used as control. After treatment cells were collected by mitotic shake-off, pelleted down and immunoprecipitation was performed by incubation with anti-Spindly antibody (see **2.7**) overnight at 4°C. Normal rabbit IgG were used as negative control. Samples were eluted in sample buffer and boiled at 95°C for 5 minutes.

Soluble fractions were resolved on Tris-glycine SDS-PAGE gels as previously described (see 2.6) and they were subsequently stained with Coomassie Instant Blue (Expedeon) for at least one hour. Bands were cut and the gel pieces were washed twice with 100 $\mu$ L 100mM Ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ): 100% Acetonitrile (ACN) for 10 minutes at room temperature on a shaker. Subsequently de-staining was performed by addition of 50 $\mu$ L of 100% ACN. Then, 100mM  $\text{NH}_4\text{HCO}_3$  was added to make 100mM  $\text{NH}_4\text{HCO}_3$ : 100% ACN and samples were incubated at 37°C for 30 minutes on a shaker.

Samples were reduced by adding 10mM of DTT and consequent boiling for 30 minutes at 50°C. For the alkylation step iodoacetamide (IAA) (Sigma) (50mM) was added following incubation in the dark at room temperature for 45 minutes. Gel pieces were dried completely in a vacuum centrifuge (maximum temperature: 45°C). The digestion was performed with 10 $\mu$ L of 1 $\mu$ g/ $\mu$ L trypsin in 490 $\mu$ L of 50mM  $\text{NH}_4\text{HCO}_3$ . 10-20 $\mu$ L of diluted trypsin solution was added to dry gel pieces and tubes were incubated overnight at 37°C. 10-20 $\mu$ L of trypsin was then added again and incubation was carried on for a further 4 hours. An equal volume of 100% ACN was supplemented to the samples and sonication was carried out for 15 minutes in a sonication bath. Supernatant was placed in a new tube and 100 $\mu$ L 70%ACN: 0.1% trifluoroacetic acid (TFA) was added. Sonication was carried out as before. Pooled supernatant volume was reduced to approximately 60-100 $\mu$ L in a vacuum centrifuge at 60°C. Excess of salt and polymers were removed from the samples by cleaning through a 'home-made' C18 "Ziptip" column (POROS R2 Applied biosystems 1-1129-06, method: <http://greproteomics.lifesci.dundee.ac.uk/webpage%20front%20page/dreamweaver%20webpage/Ziptip%20Protocol1.pdf>). Activation of the columns was carried out by washes with 20  $\mu$ L of a ACN: TFA solution (50% ACN:0.1% TFA).

The excess of 50% ACN: 0.1% TFA was washed off using 0.1% TFA and columns were loaded with the sample. The peptide solution was then loaded onto the column, and excess salt and contaminants washed away with ~40 µl 0.1% TFA. Bound peptides were eluted in a new Eppendorf tube with 40 µl of 50% ACN: 0.1% TFA. The elution step was repeated for a final volume of 80 µl. Samples were dried in a vacuum centrifuge system at 60°C to a final volume of 10 µl. This volume was then reconstituted to 30 µl with 0.1% TFA.

Peptide samples were analysed on an Orbitrap Velos (Thermo Scientific) mass spectrometer with a gradient of 2-40% Buffer A (0.1 % formic acid): Buffer B (80 % acetonitrile, 0.1 % formic acid) over 156 minutes with a flow rate of 0.3µl/min. The parent ion scan was set at a resolution of 60,000 (units) while the MS/MS was set at 'Normal scan' with a peak width of 0.6. Scans were undertaken at 335-1800 *m/z* range and the MS/MS had a minimum signal of 5000 ions. The mass window tolerances were set to 10 ppm for all data dependant acquisition. For the mass spectrometry analysis the top abundant 15 precursor ions were selected for following MS/MS, using collision induced dissociation (CID), on ions 2+ and over (1+ ions excluded) with a collision energy of 35% and an activation time of 10 milliseconds.

A 10 ml fraction of the 30 ml peptide solution was loaded on to the instrument for each run. Mass spectrometry .raw files were processed using MaxQuant 1.5.1.2.8 (Cox and Mann, 2008), and then using a Uniprot Human Database (January 2015) to identify peptides. The fixed search parameters were Carbamidomethyl (C), with variable modifications set to Oxidation (M), Phospho (STY) Sites, Acetyl (Protein N-term). Peptide spectral matching FDR (False Discovery Rate), Protein FDR and site FDR were all set to 0.01. Minimum peptide length was 7 amino acids. The data were culled using the reversed and probable contaminants indicators in the output of MaxQuant.

## 2.10 Immunofluorescence

PFA 4% solution was obtained by diluting Paraformaldehyde 32% Solution (VWR) in PHEM 1X (60mM PIPES, pH 6.9; 25 mM HEPES; 10 mM EDTA; 2mM MgCl 2x 6H<sub>2</sub>O).

Cells were seeded onto sterilised glass coverslips (Menzel-Glaser). When confluent, cells were fixed in 4% PFA for 10 minutes at room temperature, washed in PHEM-wash (PHEM + 0.1% Triton X-100), permeabilized in PHEM-T (PHEM + 0.5% Triton X-100) for 5 minutes and fixed again in 4% PFA for 10 minutes. After three washes in PHEM-wash they were blocked in Abdil (TBS-0.1% Tween, 0.1% Azide, 2% BSA) for 1 hour. At this point slides were incubated with primary antibodies for one hour at room temperature or overnight at 4°C. The antibodies were diluted in AbDil and diluted as indicated in **Table 2.2**.

**Table 2. 2. List of antibodies used.**

ANTIBODY	MANUFACTURE	SPECIES	DILUTION
Spindly	(Griffis <i>et al.</i> , 2007)	Rabbit	1:1000
DIC	(Vaughan and Vallee, 1995)	Rabbit	1:100
GFP	Novus Biological	Sheep	1:500
p50	BD Biosciences	Mouse	1:100
p150	SantaCruz	Mouse	1:100
DHC	SantaCruz	Rabbit	1:100
Tubulin	Sigma	Rat	1:500
PhalloidinAtto488	Sigma		1:500
pMyosin	Cell Signalling	Rabbit	1:200
LightChain2			

After four washes in PHEM-wash, the coverslips were incubated for one hour with secondary (either Alexa or Jackson) antibodies diluted in Abdil (1:500) and the DNA was stained with DAPI (Sigma) (1:500). Coverslips were washed three times in PHEM-wash and once in PHEM 1X prior to be mounted onto VWR SuperPremium Microscope Slides. As mounting media we used DAKO (Agilent Technologies). Slides were nail polished and let dry before imaging was performed.

High-resolution images were collected with an imaging system (DeltaVision Restoration; Applied Precision) using a 20X/0.75, 40X/1.42 oil, 60X/1.42 oil or a 100X/1.35 oil (Olympus) objective lens. Images were then processed using OMERO software or FiJi software.

## 2.11 Rapamycin mis-localisation assay

HeLa or U2OS cells were transiently transfected with three different plasmids: one FRB-, one FKBP- and one YFP- tagged. The FKBP tag was expressed in the mCherry-Spindly plasmid while the FRB tag was expressed in the Lin11 one (a plasma membrane marker) (plasmid kindly gifted from Dr. A. Saurin, University of Dundee). The YFP tag was expressed in the plasmid for the protein that we wanted to check as Spindly interactor (plasmids donated by Dr. A. Saurin, University of Dundee). After 24 hours from transfection, we administrated rapamycin (Cayman Chemical) 4 $\mu$ M and incubate for a time between 30 minutes up to 4 hours. The rapamycin induces dimerisation of the FRB-FKBP constructs (Putyrski and Schultz, 2012) mis-localising in this case mCherry-Spindly to the plasma membrane. Subsequently we fixed cells and performed immunostaining as previously described (see 2.10). Images were analysed using the Fiji Software. Measurements were conducted by drawing a line across the two edges of a cell and measuring the fluorescence intensity for both channels (green (YFP) and red (mCherry)).

### 2.11.1 Molecular cloning

Protocol performed by Dr. Eric Griffis.

The FKBP coding sequence was inserted before the stop codon of the Spindly gene using the In-Fusion cloning method (Clontech). The mCherry-Spindly plasmid (from the DSTT service) was linearized with the following primers: TAAGCGGCCGCTCGAGTCTAGAGG and CTGTTGAGGGCACTGGGTCTCTGG; the FKBP fragment (a gift from Dr. A. Saurin, University of Dundee) was amplified with the following primers: CAGTGCCCTCAACAGGGAGTGCAGGTGGAGACTATCTCC and TCGAGCGGCCGCTTATTCCAGTTTTAGAAGCTCCACATCG in which the

underlined regions are homologous to the breakpoints in the linearized mCherry-Spindly plasmid. After the ligation reaction, colonies were screened by PCR and confirmed by sequencing, which was performed by the Sequencing Service (College of Life Sciences, University of Dundee, <http://www.dnaseq.co.uk>).

## **2.12 Wound-Healing cell based assay**

U2OS cells ( $\sim 7 \times 10^5$  cells), previously treated for 96 hours with oligonucleotides for siRNA knockdown (see 2.3) either negative control or against Spindly, were seeded into the Silicone Culture-Insert (IBIDI) and left grow for at least 24 hours. Once cells were confluent, the insert was removed and cells were washed once with fresh media before beginning the imaging. A sample of the cell population was collected for Western Blot experiment to confirm the silencing of Spindly.

For the synchronised experiments, cells were treated with hydroxyurea (HU) (1mM, Sigma) for 24 hours prior being imaged. A sample of the cell population was collected for Western Blotting to confirm silencing of Spindly and S phase synchronisation.

For the rescue experiments stable U2OS cells expressing either GFP-Spindly WT or S256A were treated with siRNA for Spindly or a non-targeting control. Subsequently doxycycline (0.1-1  $\mu\text{g/mL}$ ) was administrated overnight to induce expression of the GFP construct. Then wound closure was followed over the time. A small portion of the cell population was harvested for Western Blot experiment to control silencing and GFP expression.

Video (or images) were recorded at different time points (as indicated) using a 20X/0.45 air objective (Nikon Eclipse Ti microscope system). Images were then processed using the Fiji software. Measurements were conducted by drawing a line and measuring the distance between the two edges of the wound at the indicated time points.

Kymograph analyses were conducted using Volocity software to measure the velocity of movement over the time.

## **2.13 Scratch assay**

MEF, FB or HaCaT cells were seeded onto glass coverslip and left to grow until confluence. Then we mechanically generated a scratch by using a 200 $\mu$ L tip and we fixed the slides at different time to check for protein recruitment at the leading edge. Staining protocol used was the same aforementioned (see **2.10**).

### **2.13.1 TIRF imaging**

Imaging was performed by Dr. Eric Griffis (University of Dundee).

For visualising Spindly and microtubules cells were co-transfected with mCherry-Spindly and TagBFP2-Tubulin (a gift from Mike Davidson, Florida State University, Tallahassee, FL). Conversely, for visualising Spindly and Zyxin (for focal adhesion formation), cells were co-transfected with GFP-Spindly and RFP-Zyxin (a gift from Trina Schroer, John Hopkins, Baltimore, MD). In both experiments total internal reflection fluorescence (TIRF) imaging was performed with a Nikon Ti-Eclipse with a motorized TIRF-PAU slider, a 100X/1.45 N.A. objective, a PerfectFocus 2 focusing system (Nikon Instruments), a Neos ATOF, 100mW 561nm DPSS and 100mW 405nm and 150mW 488nm diode lasers (Coherent Inc. and Solamere Technology Group), a quad dichroic (Chroma Technology), an Evolve Delta camera (Photometrics), and appropriate emission filters mounted in a filter wheel (Chroma Technology and Nikon Imaging). Images were acquired with  $\mu$ -Manager.



## 2.14 Cell fractionation experiment

U2OS cells were grown to confluence in a 150mm petri dish, washed with chilled PBS 1X and lysed in 200 $\mu$ L of Buffer A (10 mM HEPES, pH7.9, 10 mM KCl, 1.5 mM MgCl<sub>2</sub>, 1 mM DTT, 0.5  $\mu$ g/ $\mu$ L Leupeptin, 0.5  $\mu$ g/ $\mu$ L Aprotinin, 0.5  $\mu$ g/ $\mu$ L Pepstatin, 0.1 mM PMSF, 0.34 M sucrose, 10 % Glycerol, 0.1 % Triton X-100, 1 $\mu$ L DTT, 1 $\mu$ L LPC), swirled and placed on ice for 20 minutes. Cells were then scraped, transferred to Eppendorf tube and spun down at 13,000 rpm for 10 minutes at 4°C. Supernatant was removed and collected into a new Eppendorf tube as “cytoplasmic” fraction. To the remaining pellet we added 50 $\mu$ L of Buffer A combined with 0.2  $\mu$ L benzonase nuclease (SIGMA) and 10  $\mu$ L 0.1M CaCl<sub>2</sub> and incubate at 37°C for 1 minute. Sample was then placed back on ice and supplemented with 0.2 $\mu$ L of EGTA 0.5M and incubated in ice for 5 minutes. We then spun down at 13,000 rpm for 5 minutes at 4°C. Supernatant was removed and collected into a new Eppendorf tube as “nuclear” fraction. To the remaining pellet we added 50  $\mu$ L of Buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT, 0.5  $\mu$ g/ $\mu$ L Leupeptin, 0.5  $\mu$ g/ $\mu$ L Aprotinin, 0.5  $\mu$ g/ $\mu$ L Pepstatin, 1 $\mu$ L LPC) and incubated at room temperature for 1 minute. We finally spun down at 15,000 rpm for 5 minutes at 4°C. Supernatant was removed and collected into a new Eppendorf tube as “chromatin” fraction.

## 2.15 Yeast-Two Hybrid assay

The yeast strain used was AH109 (Invitrogen) that contains a HIS3 gene and a LacZ gene regulated by Gal4-binding sites. We grew the yeast in 5mL of YPAD media overnight at 30°C under constant agitation and the following morning we inoculated 50mL of YPAD with the overnight culture and let it grow until optical density (OD<sub>660nm</sub>) reached 0.6-0.8. At this point, we spun down the yeast for 3 minutes at 5000 rpm and washed the pellet with sterilised water (10mL); we spun them again (3

minutes 5000 rpm) and then re-suspended the cells in 500 $\mu$ L TE/LiAC buffer (TE 10X: Tris-HCl 100mM and 10mM EDTA; LiAC (Lithium acetate) 1M). Yeast cells were transformed with a pAS2.6 plasmid (Invitrogen) encoding Spindly fused to the DNA binding domain of Gal4 (containing a TRP1 selectable marker) and with a pACT2.6 plasmid (Invitrogen) encoding p150 or p50 to the Gal4 activation domain (containing also a LEU2 selectable marker). For the reverse experiment we transformed the yeast with Spindly fused to the Gal4 activation domain and p150/p50 fused to the DNA-binding domain of Gal4. Empty vectors were used as control. All constructs were synthesised from the Division of Signal Transduction Therapy Unit (DSTT, University of Dundee).

Competent cells were transformed with 1  $\mu$ g of DNA, 10  $\mu$ g of ssDNA salmon sperm (Invitrogen), 600  $\mu$ L of PEG/LiAc solution (polyethylene glycol/lithium acetate: 40% PEG, 1X TE, 1X LiAc) and incubated at 28°C for 30 minutes under constant agitation. We then added 70  $\mu$ L of DMSO and incubated again for 15 minutes at 42°C under constant agitation. After 10 minutes on ice and a spin down, we plated cells onto minimal medium lacking leucine (LEU), tryptophan (TRP) and histidine (HIS) and incubated at 30°C. Single colony from each transformant was grown in 5mL of minimal media (DOA, lacking histidine (HIS), leucine (LEU) and tryptophan (TRP)) overnight. The following day cells were seeded onto plates containing different concentrations of 3-amino-1,2,4-triazole (3AT) to assay reporter gene activation. 3AT is a competitive inhibitor of the product of HIS3 gene that catalyses the production of histidine. Under this condition the yeast survives only if can produce histidine, so upon binding between the bait and the prey.

To further validate positive interaction, we screened as well with 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal) that can be cleaved only in presence of an active  $\beta$ -galactosidase, producing characteristic blue colonies. We seeded cells onto

nitrocellulose membrane placed on a YPAD plate for 24 hours and then we froze the membrane in liquid nitrogen and placed it on a filter paper impregnated with XGal dissolved in DMF (Dimethylformamide) and in Z buffer ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , KCl,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , adjusted at pH=7) supplemented with  $\beta$ -mercaptoethanol. The plate was incubated at 37°C and blue colony formation was assessed for a period of 24 hours.

### 2.15.1 Site-direct mutagenesis

The vectors encoding for Spindly (both the activating, pACT2.6, and the binding, pAS2.6, plasmids) were mutated in the S256 site using Phusion Hot Start DNA polymerase (ThermoScientific). Primers used were: fwd: GTAATAGGCAACGCTTTGTTTGCAGAGGTGGGAAGATCGAAGG; rev: GGAAACAAAGCGCTTGCCTTTACTATTGGGATCCAAGGCTTGC. DNA constructs were verified by DNA sequencing performed by the Sequencing Service (College of Life Sciences, University of Dundee, <http://www.dnaseq.co.uk>).

## 2.16 *In vitro* binding assay

This experiment was carried out in the R. Vale Lab (University of California, San Francisco) using our constructs to express hSpindly WT or S256A mutated. hSpindly constructs were expressed in bacteria and protein expression was induced with 1 mM IPTG while dynein and dynactin complexes were isolated from fresh pig brains. Protein purification was carried out as described in (McKenney *et al.*, 2014) as well as the binding assay.

**3 . Spindly plays a role in the  
recruitment of the  
dynein/dynactin motor  
complex to kinetochores**

### 3.1 Introduction

When a cell enters mitosis, a high-fidelity machinery is activated to ensure proper distribution of the genomic material to the new daughter cells and also to avoid chromosome mis-segregation, which would lead to developmental disorders or cancer generation. For accurate cell division the machinery that aligns and separates the genomic material must be able to physically interact with chromosomes. One way in which this occurs is thanks to a large multiprotein structure present on chromosomes, the kinetochore (KT), that associates with spindle microtubules and allows for chromosome capture, oscillations, alignment and eventual segregation (Cheeseman, 2014).

Recruitment of the motor dynein to KTs is a critical step for proper cell division; it is central for both chromosome alignment and mitotic checkpoint (or SAC) silencing (King and Schroer, 2000) (Yang *et al.*, 2007) (Howell *et al.*, 2001). As widely described earlier (see 1.4), dynein is the sole processive minus-end directed microtubule motor in many eukaryotes, so it mediates a wide array of functions: the streaming of SAC components from KTs towards poles, the sliding of kinetochores towards the microtubule minus-ends and the proper KT-MT interactions (Allan, 2011) (Howell *et al.*, 2001) (Yang *et al.*, 2007). Upon KT-MT initial lateral interaction, dynein generates a critical pulling force that allows chromosome poleward movement; inhibition of dynein expression upon injection of anti-dynein antibodies represses chromosome movement and delays mitosis (Yang *et al.*, 2007).

The process through which the motor complex gets recruited to KTs is still under investigation, but it has been clearly proven that several kinetochore proteins can promote it. It was demonstrated that the recruitment of Spindly to kinetochores is critical as well as the presence of the RZZ complex (Barisic *et al.*, 2010) (Gassmann *et al.*, 2008) (Chan *et al.*, 2009). Initially it was hypothesised that the recruitment occurred

via the dynactin subunit p50/dynamitin, which was shown to interact with ZW10 (Starr *et al.*, 1998); however further research called this conclusion into question. Former studies have demonstrated that Spindly-depleted cells are not able to recruit the dynein/dynactin motor complex to KTs even in presence of the RZZ complex, with a consequent delay in mitosis progression due to errors in chromosome alignment and SAC silencing (Griffis *et al.*, 2007) (Gassmann *et al.*, 2008) (Chan *et al.*, 2009) (Gassmann *et al.*, 2010) (Barisic *et al.*, 2010) (Cheerambathur *et al.*, 2013). Additionally, the analysis of Spindly protein sequences identified an interesting conserved region of primary amino acid sequence lying in a break between N-terminal coiled-coil domains (called the ‘Spindly box’, in recognition of this new protein domain (Griffis *et al.*, 2007)). It was shown that a single mutation within this sequence could separate these two functions generating a protein retained onto kinetochores and unable to recruit dynein/dynactin, but still able to promote chromosome alignment (Gassmann *et al.*, 2010). In Spindly-mutant cells, the SAC was shown to be hyper-activated and SAC proteins retained on aligned kinetochores, suggesting that the removal of Spindly is essential for SAC silencing (Gassmann *et al.*, 2010).

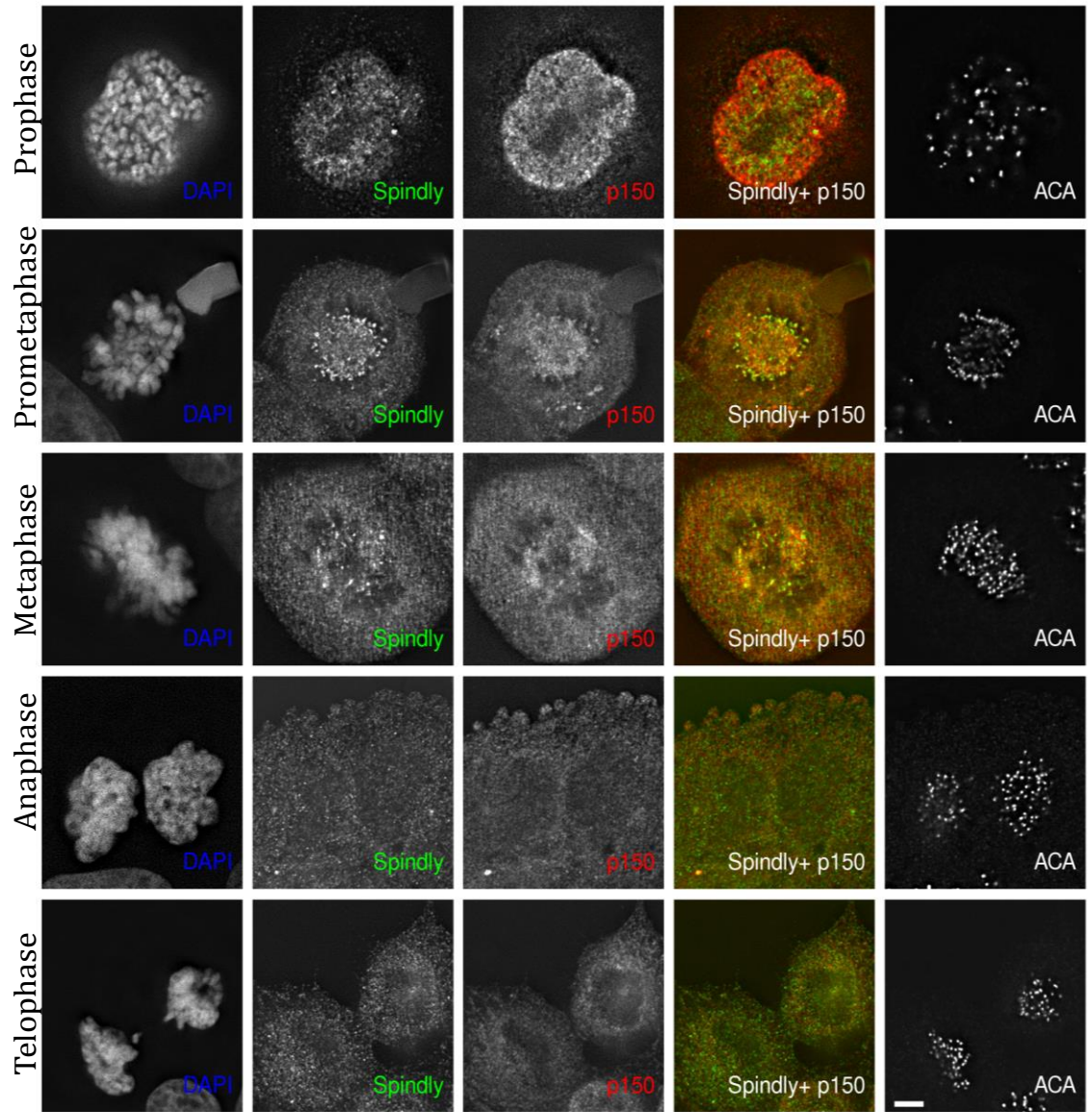
In this chapter we demonstrate that Spindly can bind the dynein/dynactin motor complex, precisely via interaction with a component of the multi-subunit dynactin sub-complex (named p150). This interaction could be needed to enhance the processivity of the motor, in line with recent publications that demonstrated that to be active and processive *in vitro* dynein requires other regulatory factors besides dynactin (McKenney *et al.*, 2014) (Schlager *et al.*, 2014). Furthermore, here we show that the mutation within the ‘Spindly box’ specifically prevents the binding of Spindly to the p150 subunit of dynactin, and, in turns, impedes the streaming of SAC effectors towards spindle poles inhibiting therefore the silencing of the checkpoint.

Overall, the results collected in this chapter describe Spindly as a novel adaptor of the minus-end dynein/dynactin motor complex important for the transport of specific mitotic cargos.

### 3.2 Spindly localises with dynactin at kinetochores

Only a few proteins are constitutively localised at centromeres throughout the cell cycle (the constitutive centromere-associated network or CCAN). Most kinetochore proteins are recruited to the centromere when mitosis begins and/or throughout mitosis. For example, the Ndc80 complex and the chromosome passenger complex (CPC) complex accumulate at centromeres in prophase, while the checkpoint effectors and the minus-end directed motor dynein localise there only in early prometaphase (Liu *et al.*, 2006). Once a cell has entered metaphase the levels of some proteins diminish (like for the SAC proteins), while instead other proteins will only then associate with kinetochores (such as EB1, APC and RanGAPs), reflecting correct KT-MT interactions and showing how MT-binding alters KT composition (Tirnauer *et al.*, 2002) (Kaplan *et al.*, 2001) (Joseph *et al.*, 2004). Therefore, proteins that compose this structure can be highly dynamic, and they show diverse residence times according to the stage of mitosis in which they play a role. It was previously shown that the minus-end directed motor dynein and its adaptor dynactin are among those proteins that are enriched at kinetochores only in prometaphase, and that they move to spindle poles once bi-orientation is achieved (Pfarr *et al.*, 1990) (Echeverri *et al.*, 1996). Figure 3.1 shows that Spindly has a similar distribution profile in mitosis: it gets recruited to kinetochores in early prometaphase and then, in metaphase, it starts to stream along k-fibres to reach the spindle poles. As expected, in anaphase and telophase Spindly is no longer detectable at kinetochores.



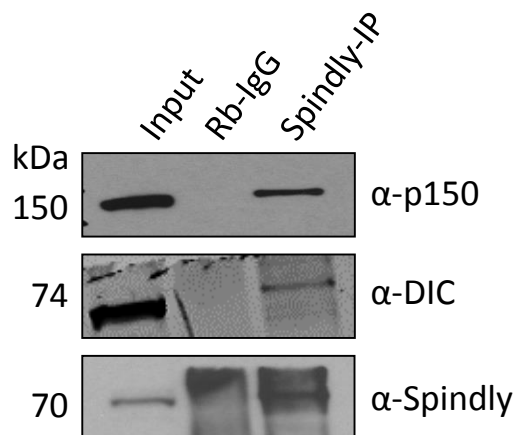


**Figure 3. 1. Mitotic distribution of Spindly**

U2OS cells were stained with anti-Spindly (green), anti-ACA (anti-centromere antibody, white) and anti-p150 (red) antibodies. Spindly localises to kinetochores during prometaphase, mainly at the spindle poles and along k-fibres in metaphase and it is no longer detectable in anaphase and telophase; similar distribution profile is reported for dynactin (p150 staining), with which Spindly co-localise. Chromosomes were stained with DAPI (blue). 100X magnification. Scale bar: 5µm. Double channel representation of either Spindly+ACA or p150+ACA is presented in the appendix, to show their kinetochore localisation (**Chapter 8- Fig. 8.2**).

### 3.3 Spindly interacts with the dynein/dynactin motor complex

Spindly was first identified as a crucial player for proper completion of mitosis; S2 *Drosophila melanogaster* cells lacking Spindly arrest in metaphase and cannot recruit the dynein/dynactin motor complex to promote the removal of the checkpoint effectors and anaphase onset (Griffis *et al.*, 2007). To date, the importance of Spindly in this process has been clearly underlined, but evidence of direct interaction between Spindly and the motor complex is still missing. With the aim to investigate this relationship more closely, we decided to perform immunoprecipitation of endogenous Spindly from HEK293 cell extracts to analyse the presence of either dynein or dynactin subunits. Figure 3.2 shows Spindly association with the p150 subunit of dynactin as well as with the dynein intermediate chain (DIC).



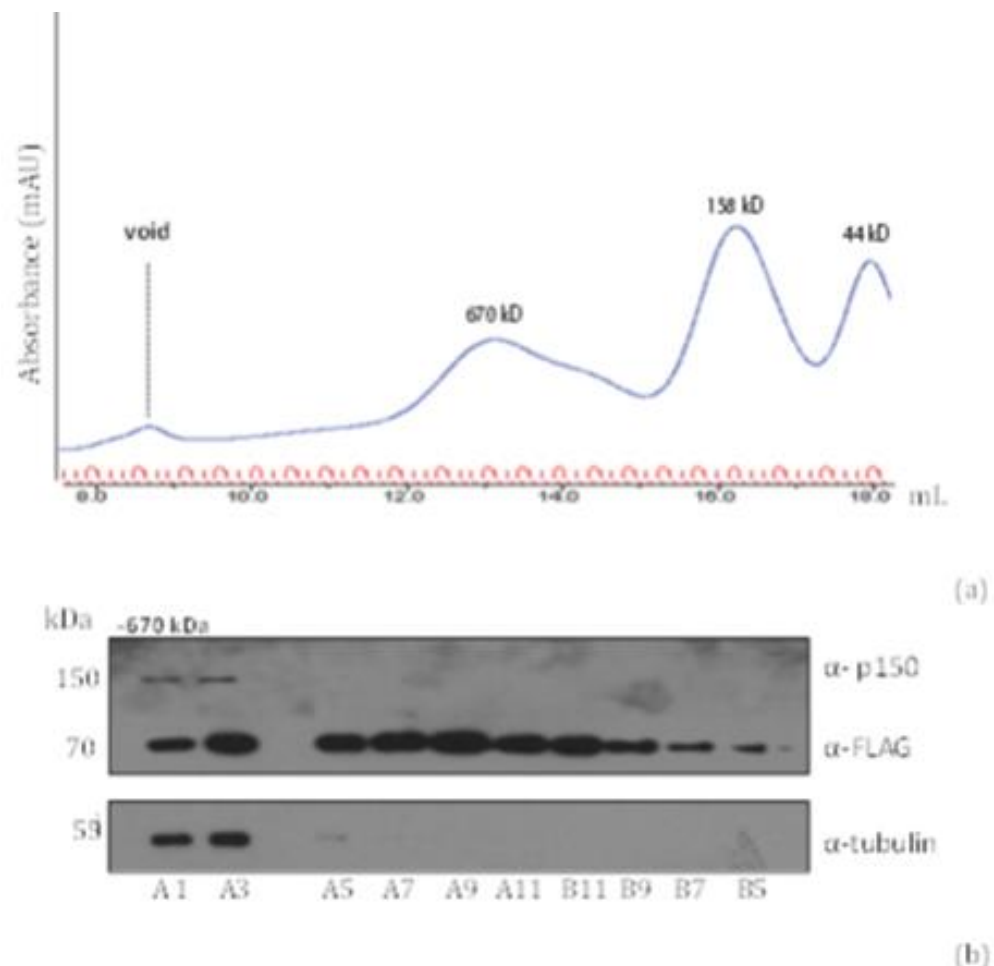
**Figure 3. 2. Spindly immunoprecipitates both dynein and dynactin.**

Immunoprecipitation of endogenous Spindly from HEK293 cell lysates. 200/400  $\mu$ g of total cell extract were used to immunoprecipitate Spindly with anti-Spindly antibody. Rabbit IgG was used as negative control. Immunoprecipitated complexes were analysed by Western blot using the indicated antibodies. Input corresponds to whole cell extract. Anti-Spindly antibody specificity was validated by testing samples treated with control siRNA or siRNA against Spindly that confirm the molecular weight band predicted for the protein (see Fig. 3.6, this chapter). (DIC: dynein intermediate chain)

Since dynein and dynactin associate closely (Vaughan and Vallee, 1995) (Echeverri *et al.*, 1996), the result obtained in Fig. 3.2 suggested that either Spindly interacts with both complexes independently, or it binds to only one of the two but

indirectly it pulls down both complexes due to their own interaction. Hence, we sought to study the elution profile of these three proteins taking advantage of size exclusion chromatography technique, with the aim of delineating the potential complex.

We performed gel filtration experiments fractionating lysates from HEK293 cells on a Superose 6 column (Fig. 3.3). Western blot analysis of the fractions revealed the presence of Spindly, as well as dynactin (p150 subunit), at the beginning of the elution profile, immediately after the void volume (Fig. 3.3, (a)), indicating the presence of a large complex that is likely to include many other proteins besides Spindly and dynactin. Indeed Tubulin had a similar elution profile (Fig. 3.3, (b)).

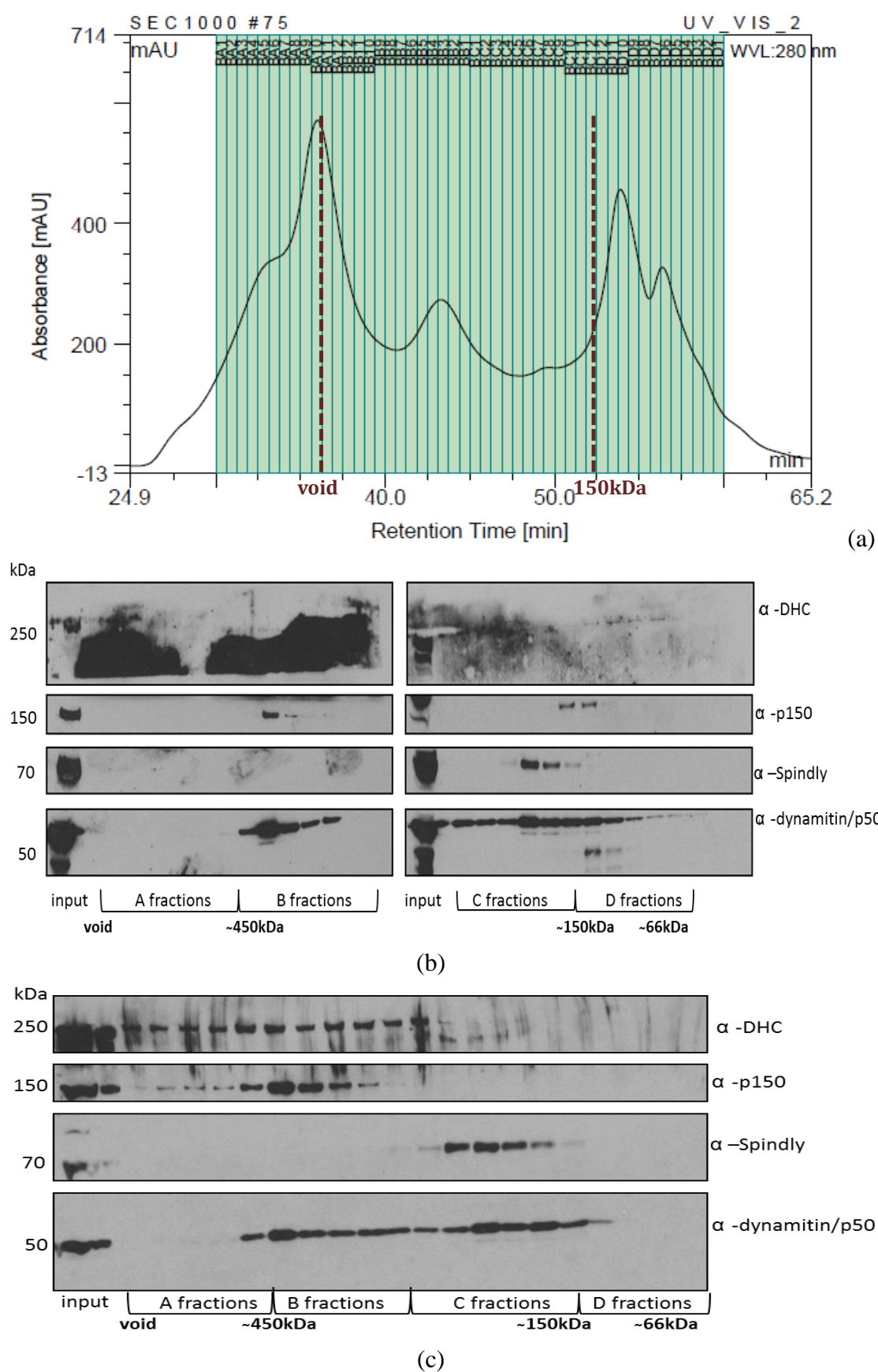


**Figure 3. 3. Spindly co-elutes with dynactin.**

Extracts of HEK293 cells expressing FLAG-Spindly were analysed by gel filtration on a Superose 6 column and resolved by Western blot. (a) Elution profile ( $A^{280}$ ) of the total cell lysate; (b) Western blot analysis of collected fractions. Antibodies as indicated.

Data in figure 3.3 indicates that with the column used we could not separate properly the complex. Indeed Tubulin is the core component of microtubules and its presence in the same fractions with Spindly and dynactin indicates the possibility that we did not isolate the different single components of the complex from the microtubules in our lysate. Therefore, we decided to carry out size-exclusion chromatography of the extracts using a SEC-1000 gel filtration column that provides greater separation of the higher molecular weight species. Cell lysates from either asynchronous (Figure 3.4, (b)) or mitotically enriched (Figure 3.4, (c)) HEK293 cells were passed through the column and then analysed by Western blot. Figure 3.4 shows the dynactin complex (p150 and p50 subunits) enriched in B fractions (~450 kDa), while Spindly elutes preferentially in the later fractions (C fractions (~250/200 kDa); Fig. 3.4. (b) and (c)).

It is worth to point out that the two diverse SEC experiments (Fig. 3.3 and Fig. 3.4) differed not only in the type of SEC column used but also in the protocol used for the lysate preparation (see **2.8**); samples analysed by size-exclusion chromatography on a SEC-1000 were sonicated and the fractionation step was conducted at room temperature.



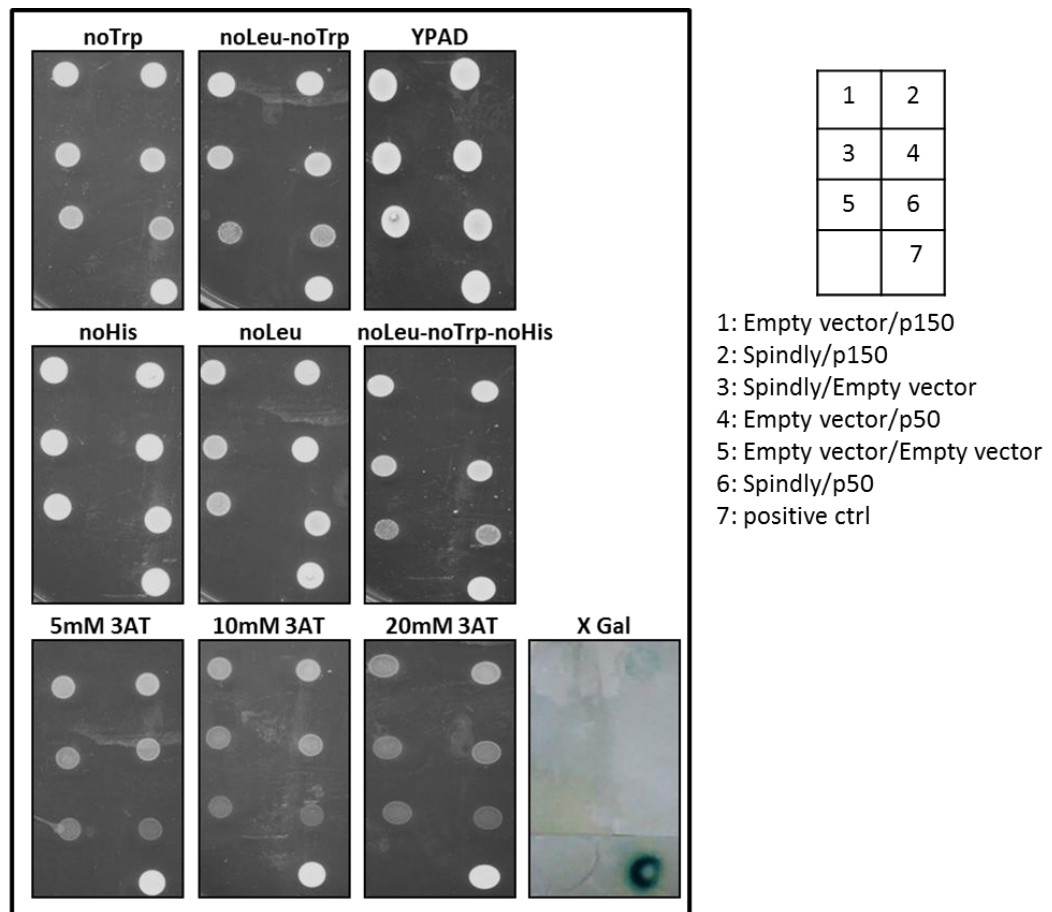
**Figure 3. 4. Spindly elution profile.**

Extracts of HEK293 cells were analysed by size exclusion chromatography on a SEC-1000 gel filtration column. Elution profile of the total cell lysate for both experiments (a); Fractions were resolved by Western blot: asynchronous cells (b), or mitotically enriched cells (c). Antibodies as indicated. Inputs represent the whole cell lysate. (DHC: dynein heavy chain)

This result pointed out that Spindly can be a part of two different complexes: one with the dynactin complex (a heavier complex eluting after the void volume, see Fig. 3.3) and another one with other potential partners that would need to be further explored (Fig. 3.4). Also it is important to mention that Spindly is a very elongated molecule and that we have seen, from the purification of recombinant proteins, which it does not run according to its molecular weight in size-exclusion chromatography experiments (data not shown). Both the rod-like shape of Spindly structure together with the high presence of coiled-coil regions could be affecting its running properties, suggesting possible presence of dimers instead of monomers of the protein.

### **3.4 Spindly associates directly with the p150 subunit of dynactin**

Data collected so far led us to believe that there could be an interaction between Spindly and dynactin, but one that is not stable enough to last through gel filtration. To confirm this, we decided to carry out a yeast-two hybrid assay using two dynactin subunits, p150 and p50 as baits and Spindly as a prey (or vice versa). To this aim, bait/prey constructs were generated (from the DSTT unit, University of Dundee) containing either a GAL4 DNA-binding domain or GAL4 DNA-activating domain. Transformed cells were then plated onto minimal medium lacking leucine (LEU), histidine (HIS) and tryptophan (TRP) to assay reporter gene activation. Selective media contained different concentrations of 3 AT (3-amino-1,2,4-triazole), a competitive inhibitor of the product of HIS3 gene. Under this condition the yeast will survive only if it can produce histidine, therefore only upon binding between the bait and the prey that will activate the transcription of the reporter gene. Moreover, to test positive interaction, cells were plated onto nitrocellulose filter paper that was frozen in liquid nitrogen and then placed onto a piece of filter paper impregnated with X-Gal (5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside) to test for activity of  $\beta$ -galactosidase in cleaving its substrate lacZ to generate blue colonies (Fig. 3.5).



**Figure 3. 5. Spindly interacts with the p150 subunit of dynactin.** Yeast two-hybrid assays were performed with a GAL4 DNA-binding domain fusion (or activation domain fusion) for each protein as indicated in the table on the right hand-side of the panel. Cells were grown on media lacking LEU, TRP and HIS and selected for histidine production by adding different concentrations of 3-AT or tested for lacZ reporter gene activity (XGal). Empty vectors were used as control. Positive control used were SLX4 (activating domain) and XPF (binding domain).

Experiment in figure 3.5 revealed that only when the Spindly construct was mixed with the p150 construct, cells grew on the selective media and promote LacZ activity; they instead did not show the same ability when Spindly and p50 constructs were mixed. Since Spindly is absent from yeast and the p150 construct was not active by itself, we can conclude that there is likely a direct association between Spindly and p150. However, it is important to point out that, although Spindly/p150 colony seemed to be the densest one, in the data presented the empty vector controls did show potential



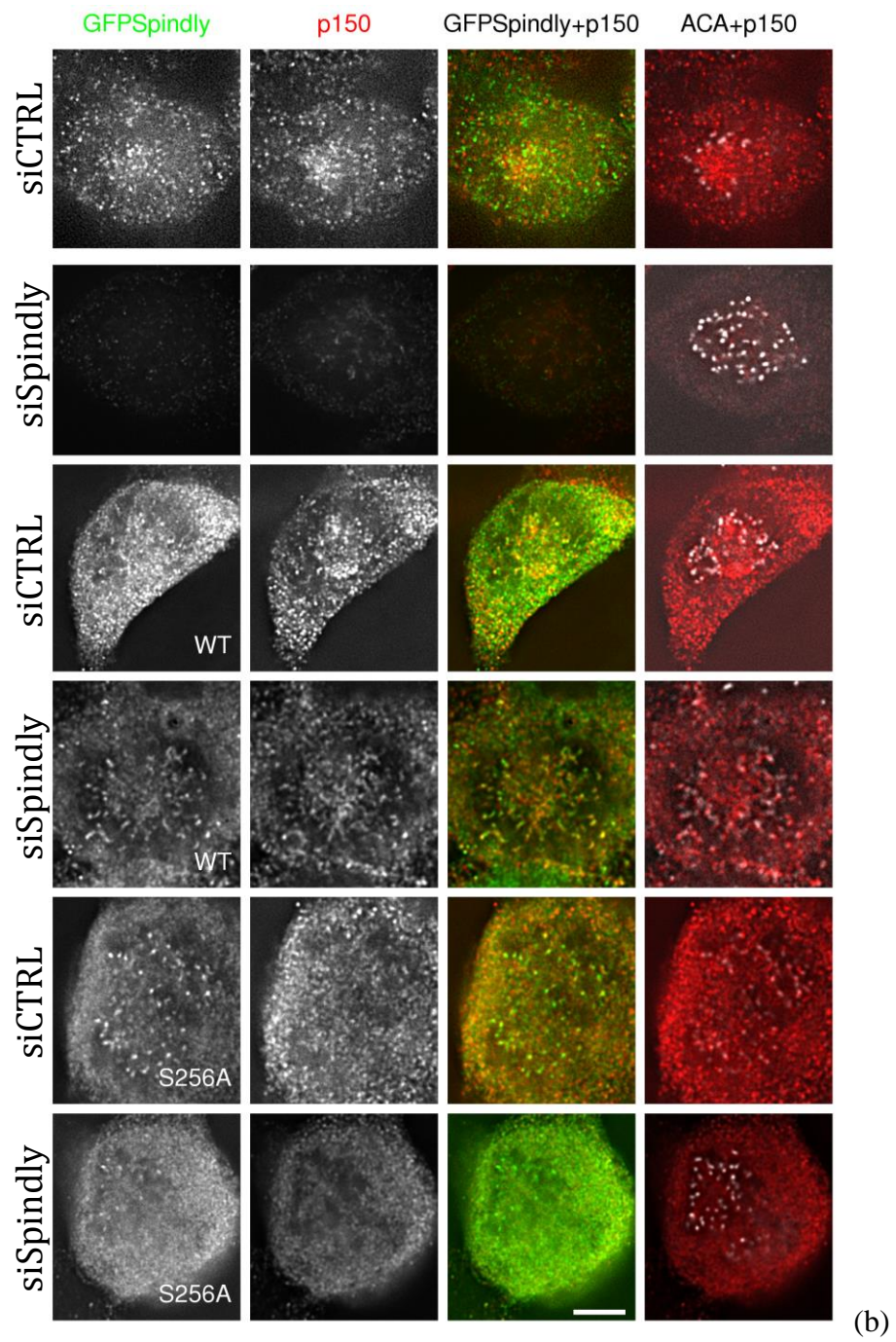
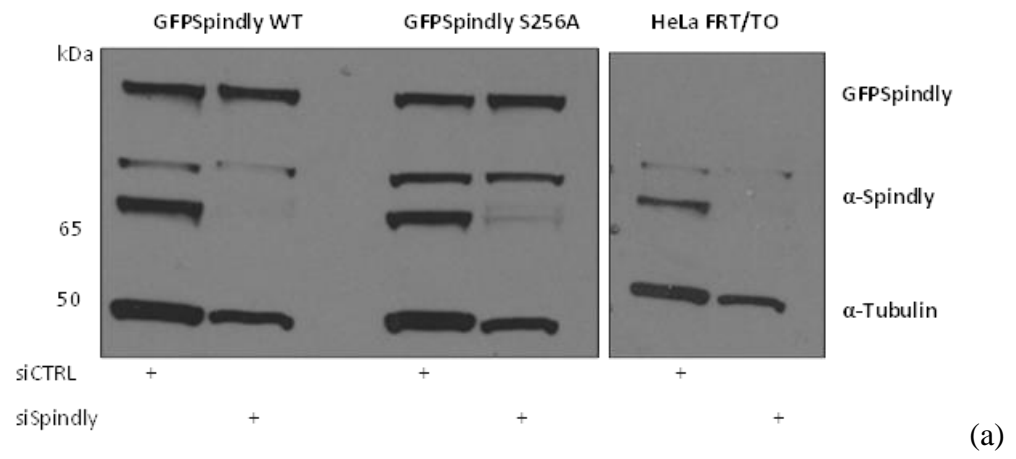
cross-activation (basal growth visible), requiring further validation to prove the specificity of the interaction.

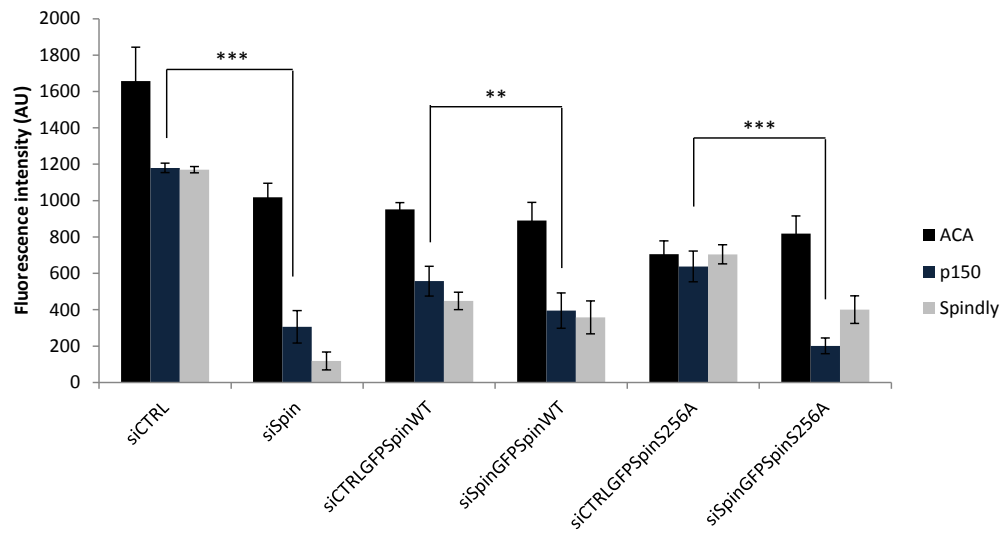
### **3.5 The interaction with dynactin relies on a specific region within Spindly**

#### **3.5.1 Substitution in the Spindly box affects kinetochore recruitment of p150**

Spindly was identified in *D. mel.* as a critical recruiting factor of dynein but not of dynactin at KTs (Griffis *et al.*, 2007). Conversely, human Spindly was described as crucial for the recruitment of the whole motor complex and therefore it was proposed that it could be binding either of the multi-subunits of the complex. Subsequently, Gassmann and colleagues identified two single point mutations within the ‘Spindly box’, the conserved region present in all Spindly proteins, that could affect the capacity of Spindly to recruit dynein/dynactin to kinetochores (Gassmann *et al.*, 2010) (Fig. 3.6 (d)). Substitution of Serine 256 (or Phenylalanine 258) with Alanine produces a Spindly protein that, even if still able to bind to KTs, totally prevents the silencing of the checkpoint and leads to metaphase-arrest of cells (Gassmann *et al.*, 2010).

We therefore wondered if this single point mutation could disturb the direct binding of Spindly to the motor complex and, more precisely, the association with p150, which we described above. Hence, we decided to analyse the recruitment of dynactin to kinetochores upon expression of Spindly mutant. To this end, we performed immunostaining of HeLa cells depleted of the endogenous Spindly, but expressing GFP Spindly WT or S256A under stimulation with doxycycline (Fig. 3.6 (a) (b)).





(c)

A  
↑

<i>H. sapiens</i> 1-605	251	NSKGNSLFAEVEDRRAA	267
<i>M. Musculus</i> 1-608	251	NSKGNSLFAEVEDRRVA	267
<i>G. gallus</i> 1-608	251	TSKGNSLFAEVEDRRAE	267
<i>X. laevis</i> 1-610	252	NSKGNSLFAEVEDRRSE	269
<i>D. rerio</i> 1-590	253	NSKGNSLFSEVEDKRAA	269
<i>S. purpuratus</i> 1-564	206	NKKGNSLFAEVEDKRQA	222
<i>D. melanogaster</i> 1-807	229	DRKGNSLFAEVDQQRQA	245
<i>C. elegans</i> 1-479	193	AARGNSMFSEVIDAERK	208

(d)

### Figure 3. 6. Single point mutation affects the recruitment of the motor complex.

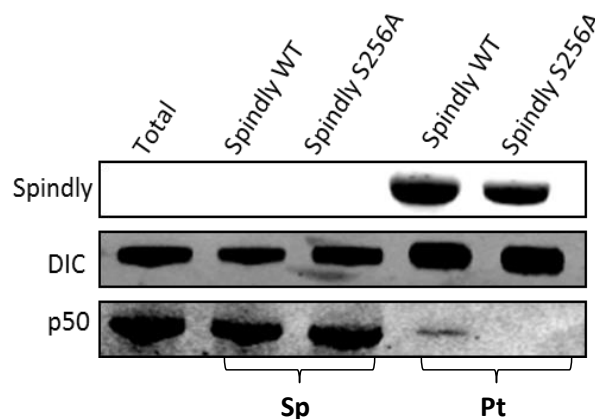
HeLa cells expressing indicated GFP-Spindly constructs were incubated for 96 hours with siRNA to deplete endogenous Spindly expression. To drive the expression of GFP-Spindly, doxycycline was administrated to a final concentration of 1µg/mL for a minimum 24 hours. (a) Western Blot confirmed Spindly silencing and GFP expression upon administration of doxycycline (1 µg/mL). (b) Immunofluorescence of HeLa cells expressing different Spindly constructs; staining shows: nuclei (DAPI, blue), Spindly (either endogenous or GFP tagged, green); dynactin (p150, red); kinetochore (ACA, white). 100X magnification. Scale bars: 5µm. (c) Graph shows relative fluorescence intensity levels of kinetochore recruitment of p150, Spindly and ACA. Fluorescence intensity at kinetochores was measured for different channels using the FiJi software (n ≥ 100). Mean ± standard deviation (represented by error bars) were determined from at least three independent experiments. Statistical analysis was performed with Excel and data were analysed by Student's *t-test* with significance defined by \*: \*\*= p< 0.005; \*\*\*= p<0.001. (d) Sequence alignment of the conserved motif in the Spindly protein family. Red arrow indicates the conserved Serine (S) that was mutated to Alanine (A) for the experiment.

Quantifications of immunofluorescence images (Fig. 3.6 (b) and (c)) confirmed defects in the kinetochore enrichment of dynactin (p150 subunit staining) when Spindly was

mutated within the ‘Spindly box’ (S256A) (Fig. 3.6 (c)), corroborating our hypothesis of a defective binding with dynactin upon mutation. Defects in p150 KT-association was highly specific for dynactin; indeed when we tested dynein recruitment we did not registered any defect upon Spindly depletion (data not shown).

### 3.5.2 Expression of Spindly mutants shows defective binding only with dynactin

To shed light on this relationship we collaborated with the Vale lab (namely with Dr. Richard McKenney, University of California, San Francisco) in order to test, by an *in vitro* binding assay, the capacity of Spindly, either WT or S256A, to bind dynein and dynactin. Spindly was purified from bacteria and dynein/dynactin complexes from fresh pig brains. The proteins were mixed together and Spindly and interactors were pulled down with StrepTactin agarose beads (as described in (McKenney *et al.*, 2014). Figure 3.7 reveals that Spindly S256A can bind only with dynein (DIC) but not with dynactin (p50 subunit). This result is in line with the underlined defects in p150 kinetochore-recruitment presented in figure 3.6 (b) and (c).

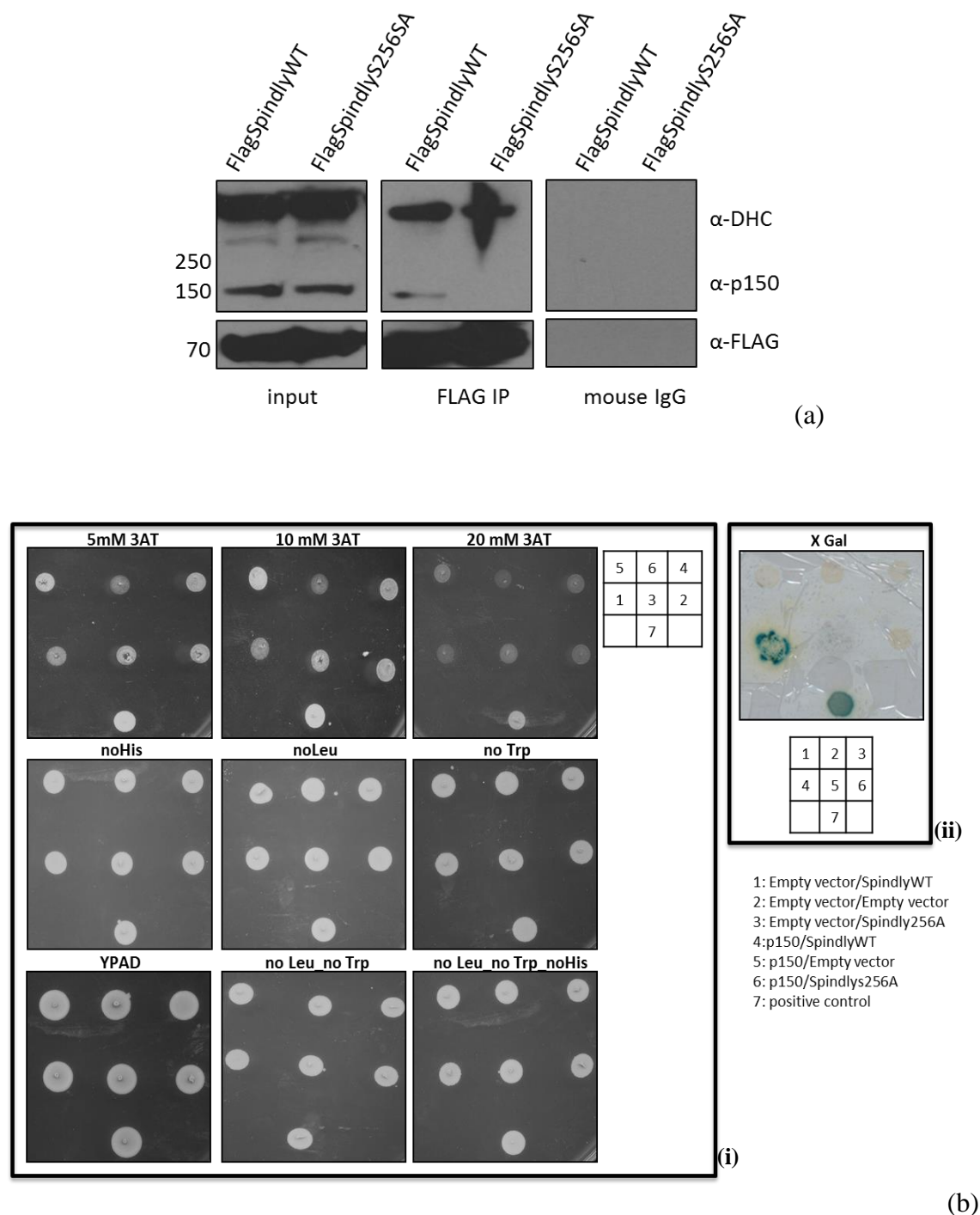


**Figure 3. 7. SpindlyS256A expression impairs the binding to dynactin.**

Recombinant full length human Spindly, WT or S256A were expressed and purified from bacteria. Dynein (DIC: dynein intermediate chain) and dynactin (p50/dynamitin subunit) complexes were separately purified from pig brains and mixed with the tagged Spindly attached to beads. The binding assay was performed as indicated in (McKenney *et al.*, 2014). Sp: supernatant; Pt: pellet. Experiment conducted in the Vale Lab by Dr. R.J. McKenney (University of California, San Francisco).

### 3.5.3 The Spindly box plays a crucial role in Spindly-dynactin association

Results above described led us to think that the mutation directly impaired the association of Spindly with dynactin. To corroborate this hypothesis, we decided to test the interaction in cells. To this aim we generated FLAG-Spindly constructs (WT or S256A) and expressed them in HEK293 cells. Using an anti-FLAG antibody we carried out a FLAG pull down. Data reported in figure 3.8 (a) confirms that the S256A mutation specifically affects the interaction between Spindly and dynactin, but it does not perturb the interaction with dynein. Furthermore, the yeast-two-hybrid assay using a Spindly S256A construct (either as a prey or as a bait), corroborate the impairment of the binding as a result of the mutation; it was possible to record positive interaction only when the yeast was transformed with the Spindly WT construct (Figure 3.8, (b)).



**Figure 3. 8. The serine 256 is crucial for Spindly association with dynactin.**

(a) HEK293 cells were transfected with the indicated constructs. 200/400  $\mu$ g of total protein was used to immunoprecipitate FLAG-Spindly from the cells. Mouse IgG was used as negative control. Immunoprecipitated complexes were analysed by Western blot using the indicated antibodies. Inputs correspond to whole cell extracts. (b) Yeast two-hybrid assays were performed with a GAL4 DNA-binding domain fusion (or activation domain fusion) for each protein as indicated in the table on the top right corner of (i) and on the bottom side of (ii). Cells were grown on media lacking LEU, TRP and HIS and selected for histidine production by adding different concentrations of 3-AT (i) or tested for lacZ reporter gene activity (ii). Empty vectors were used as control.

Putting together our data we conclude that Spindly recruits the minus-end motor complex dynein/dynactin to kinetochores by binding the p150 subunit of the dynein-activator dynactin. This association requires the ‘Spindly box’ region. Our data also revealed that Spindly S256A can still pull down dynein (Fig. 3.8 (a)), hinting to an association that in this case does not go through the ‘Spindly box’ region and that is possibly dynactin-independent.



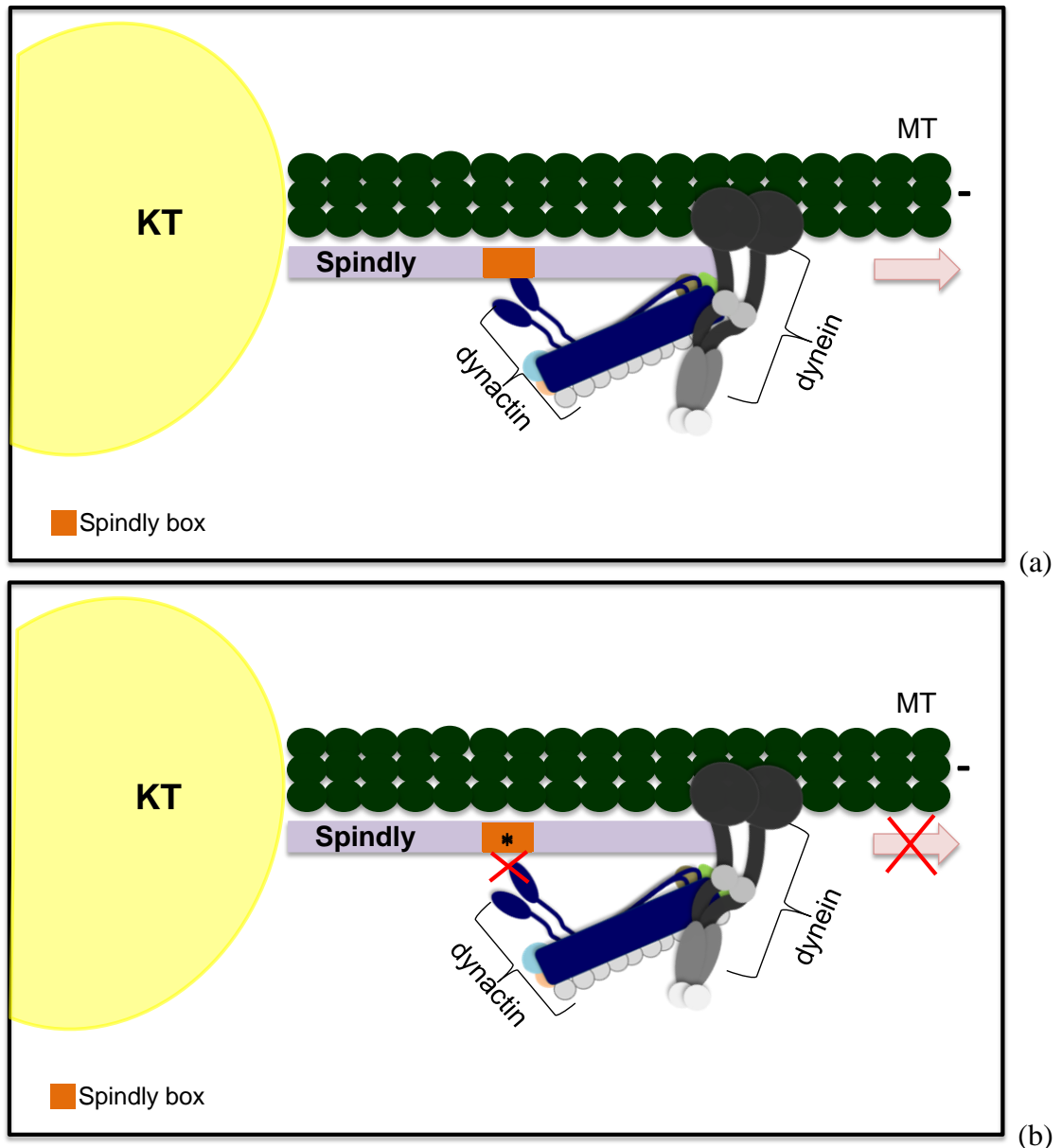
### 3.6 Discussion

The capacity of dynein to be processive is different in diverse species; *S.cerevisiae* dynein has to dimerise in order to be processive (Nishiura *et al.*, 2004) (Reck-Peterson *et al.*, 2006), while mammalian dynein has been reported to make a step 8nm long (as in the other species) only when multiple molecules of dynein interact with a microtubule, showing mainly diffusive movements when expressed by itself (Mallik *et al.*, 2004). Dynein utilises ATP hydrolysis to walk along microtubules, but it requires co-activators to promote active processive movements to reach the minus-end of a microtubule and to load cargos (Carter, 2013) (Schlager *et al.*, 2014).

Many studies have underlined the importance of dynactin for the execution of the different functions of dynein within cells (King and Schroer, 2000) (Culver-Hanlon *et al.*, 2006), but it has also been shown that it is not sufficient to change the movement rates of dynein along microtubules (Schlager *et al.*, 2014) (McKenney *et al.*, 2014). Over the years, many different adaptors that help dynein to bind specific cargos have been identified and more recent studies have pointed out how one of these, Bicaudal-D2 (BICD2), can work together with dynactin in order to promote the processivity of the motor (Kardon and Vale, 2009) (Vallee *et al.*, 2012) (Schlager *et al.*, 2014) (McKenney *et al.*, 2014). To date, dynein is the only processive minus end directed motor described in cells outside of plants, and thus the need of adaptors that promote the binding and the loading of specific cargos is of critical importance and strictly related with its high versatility.

Analysing our results we conclude that Spindly is a novel adaptor for the dynein/dynactin motor complex and hence it might help the motility of the motor along microtubules and potentially the direct loading of specific cargos. Figure 3.9 represents a schematic model of what we think could be happening: when Spindly is expressed in its endogenous wild-type form, it promotes the recruitment of the motor complex to the

kinetochore (KT); in turn the cargo is loaded onto the motor that subsequently moves along the microtubule (MT) (Fig. 3.9, (a)). Conversely, when Spindly is mutated within the ‘Spindly box’ (Spindly box\*), the binding via p150 is defective, the cargo loading is impaired and the processivity of the motor along the MT is not enhanced (Fig. 3.9, (b)). So the motor still localises at KT but it cannot be activated.



**Figure 3. 9. Schematic model of the ternary complex.**

(a) The presence of Spindly WT enhances the binding between dynein and dynactin, promoting the cargo loading and the movement towards the minus-end of a microtubule (MT); (b) on the other hand, when Spindly is mutated within the Spindly box (Spindly\*), the binding with dynactin is impaired with consequent defects in the motor processivity and in the cargo.

It is important to notice that in our study we demonstrated how Spindly S256A can still bind to dynein (Fig. 3.7; Fig. 3.8); this is in line with previous studies that described Spindly mutants still able to sustain correct chromosome alignment (Gassmann *et al.*, 2010) and allow for normal dynein streaming process from kinetochores (Dr. K. Vaughan personal communication). However, it has been revealed how instead the same mutation on Spindly abrogates dynein-kinetochore recruitment leading to chromosome segregation defects similarly to Spindly depletion phenotypes (Cheerambathur *et al.*, 2013). Consequently, to date, the mechanism through which dynein gets recruited to kinetochore is still not entirely defined. The initial model proposed, by which dynein is localised to KT's via the RZZ complex and Spindly seems to be incomplete; it appears to be not comprehensive for all of the interactions identified up to the present time. Indeed, conflicting reports have stated that either dynein requires dynactin-mediated interaction with the RZZ complex to be localised, or, in a totally opposite direction, dynein can be assembled at kinetochores in absence of the RZZ complex, binding via the factor NudE/NudEL (Starr *et al.*, 1998) (Stehman *et al.*, 2007). Obviously clarity needs to be gained, but it is definitely conceivable that there is a handoff between different recruitment factors that allows for the targeting of dynein and consequently for the execution of all its functions at the kinetochore. It also raises the possibility that some of the actions of dynein at the KT do not require processivity (namely overcoming the RZZ-dependent inhibition of KT-MT binding) and hence do not require association with dynactin, while other activities (such as SAC streaming or KT gliding) are processive and dependent upon dynactin binding. Future experiments will be needed to sort out these possibilities.

## **4 . Spindly is an active player of the Spindle Assembly Checkpoint**

## 4.1 Introduction

The Spindle Assembly Checkpoint (SAC) is the mitotic checkpoint necessary to ensure that chromosome alignment proceeds towards anaphase onset with the right timing; it senses the status of kinetochore-microtubule attachments (or tension) and generates a signal that prevents anaphase until all chromosomes are correctly aligned on the spindle (Musacchio, 2011) (Lara-Gonzalez *et al.*, 2012).

As already described (see **1.2.3**), the activation and the perpetuation of the signal are based on the presence on kinetochores of the SAC components: Mps1, Bub1, Bub3, Mad1, Mad2, Mad3/BubR1 and Cdc20 (Foley and Kapoor, 2013). The mitotic checkpoint complex (MCC: Mad2-Cdc20 plus BubR1-Bub3) prevents mitosis progression by inhibiting the ubiquitin ligase activity of the APC/C complex and preventing the degradation of Cyclin B and Securin, which respectively establish and maintain the mitotic phospho-environment and inhibit Separase to prevent the cleavage of the cohesin molecules that link sister chromatids (Musacchio, 2011) (Lara-Gonzalez *et al.*, 2012). Once bi-orientation is achieved, the SAC is silenced and the activity of the APC/C can drive anaphase onset. Several processes contribute to the silencing: disassembly of the MCC, recruitment of phosphatases to KT's and physical removal of the SAC components from KT's (Wang *et al.*, 2014). This latter step is mediated by the minus-end directed motor dynein and, as discussed in the previous chapter, its localisation is directly dependent upon Spindly recruitment at KT's (Griffis *et al.*, 2007). Indeed, any treatment that affects dynein localisation at kinetochores (such as dynein inhibition or expression of the SpindlyS256A mutant) causes retention of Spindly and checkpoint proteins at KT's (Griffis *et al.*, 2007) (Gassmann *et al.*, 2010). Conversely, elimination of Spindly function (by depletion of either Spindly itself or a component of the RZZ complex) allows for SAC silencing even without dynein recruitment to KT's

(Gassmann *et al.*, 2008). All together these previous reports indicate that Spindly could play a function in SAC activation and/or maintenance.

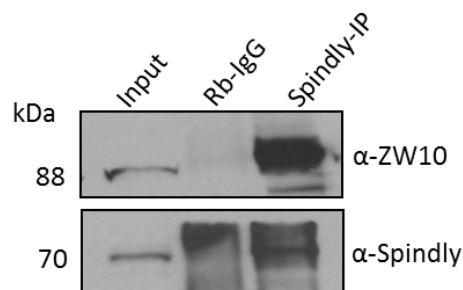
In this chapter a crosstalk between Spindly and some of the components of the mitotic checkpoint is shown. We identified novel mitotic binding partners of Spindly directly involved in SAC signalling or assembly (Mps1, BubR1, CENP-E and CENP-F). We also described an interaction between Spindly and PP2A, a phosphatase recently demonstrated to be a key player in the checkpoint silencing process that could suggest how the cell coordinate the SAC inactivation step (Espert *et al.*, 2014) (Nijenhuis *et al.*, 2014).

Overall, data here presented indicate that Spindly, apart from a function in mitotic checkpoint silencing, acts in maintaining the checkpoint signalling mostly via its association with BubR1.

## 4.2 Spindly binds kinetochore via the RZZ complex

The assembly of the spindle checkpoint relies on kinetochores (Foley and Kapoor, 2013). As described in the introduction (see 1.2.2), the kinetochore contains an outer plate on which several proteins are rapidly assembled at the beginning of mitosis, where the mitotic checkpoint effectors are recruited (Joglekar *et al.*, 2006) and the RZZ (Rod-Zwilch-Zw10) complex ((Karess, 2005), and Spindly exert their function (Griffis *et al.*, 2007).

A recent paper from Varma and co-workers has depicted Spindly as a long rod-shaped protein that binds kinetochores via its C-terminal region by interacting with the RZZ complex (Varma *et al.*, 2013). To confirm this idea, we performed immunoprecipitation experiments using HEK293 cells, mitotically enriched by overnight treatment with S-trityl-L-cysteine (STLC; a specific inhibitor of the kinesin Eg5 that induces monopolar spindles and arrests cells in M phase (Skoufias *et al.*, 2006)) and then released for two hours into Colchicine (an inhibitor of microtubule polymerisation (Taylor, 1965)). Mitotic cells were collected by shake-off and immunoprecipitation was then carried out by incubating cell lysates with an anti-Spindly antibody overnight (Fig. 4.1). Results from this pull-down corroborate the hypothesis that Spindly binds to the RZZ complex (Fig. 4.1).



**Figure 4. 1. Spindly interacts with the RZZ complex.**

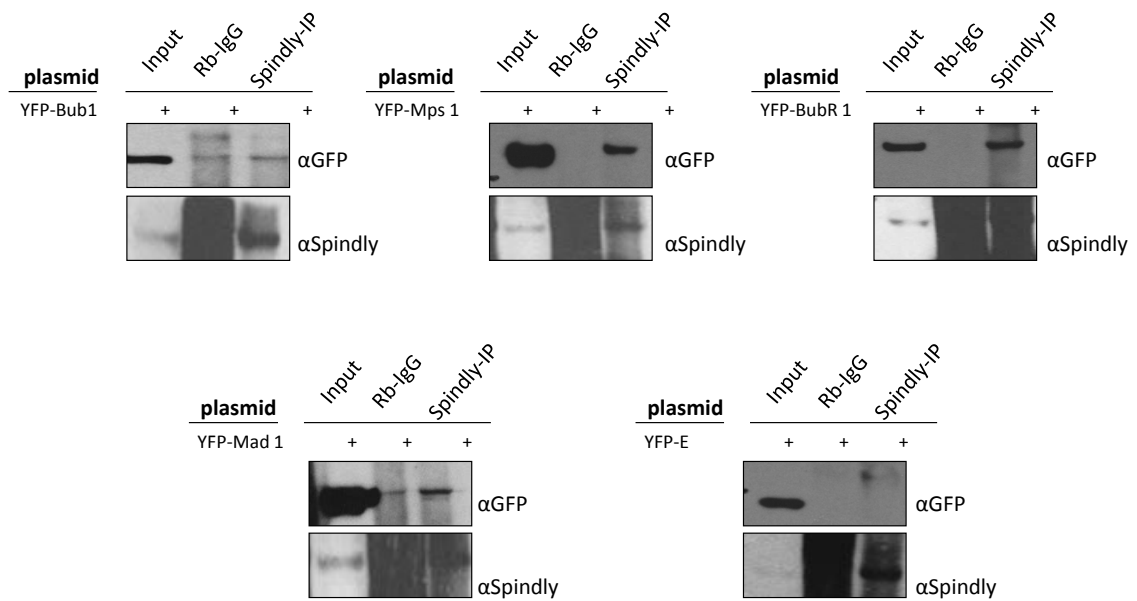
HEK293 cells were enriched in mitosis and immunoprecipitation was performed with anti-Spindly antibody. Rabbit IgG (Rb- IgG) were used as negative control. Samples were blotted with indicated antibodies. Input represents the whole cell lysate. Samples used in this experiment have already been presented in this thesis (see Fig. 3.2).

### 4.3 Crosstalk between Spindly and the SAC molecular players

The RZZ complex plays a role in the recruitment of Mad1/Mad2 complex and consequently also in SAC activation (Buffin *et al.*, 2005) (Kops *et al.*, 2005). Given the interaction between Spindly and ZW10, we wondered whether KT recruitment of Spindly could be involved as well in the assembly or amplification of the whole checkpoint signalling. Hence, it was decided to study the interaction between Spindly and different SAC effectors. To this end, HEK293 cells were transfected with BubR1, Bub1, Mad1 or Mps1 - YFP expression vectors, or an empty vector (YFP-E) and, after 40 hours from transfection, we mitotically enriched the cell population by adding Nocodazole (an inhibitor of microtubules polymerisation (Spurck *et al.*, 1986)) overnight. Cells were subsequently harvested by mitotic shake-off and immunoprecipitation was performed with anti-Spindly antibody (Fig 4.2).

Figure 4.2 indicates that there is an association of Spindly with BubR1 and Mps1, while results were not very convincing for interactions with Mad1 and Bub1. Data obtained substantiates the starting hypothesis that Spindly is involved within the spindle assembly checkpoint pathway.





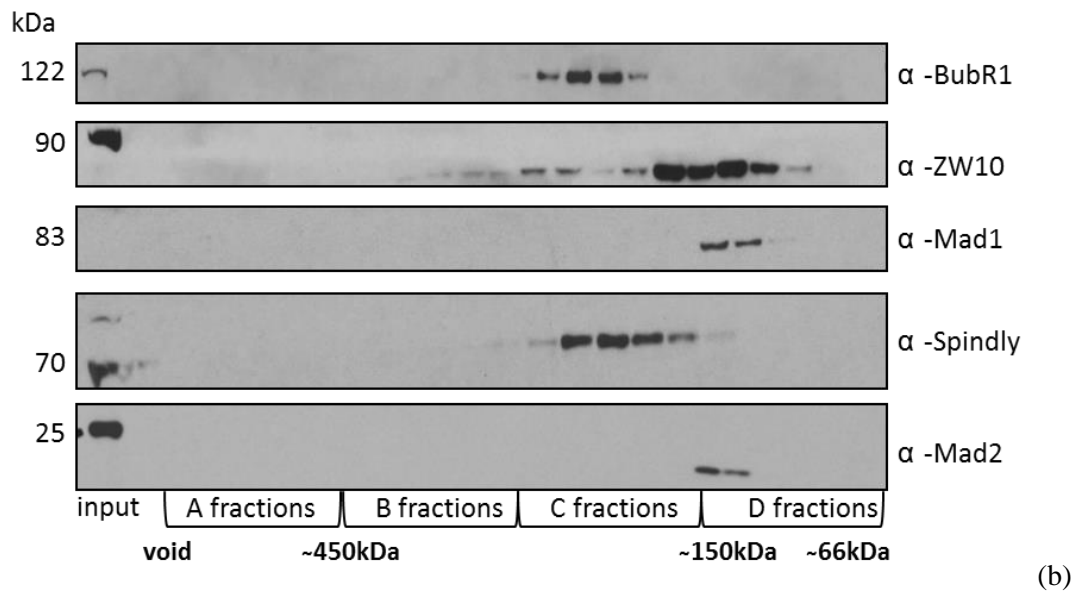
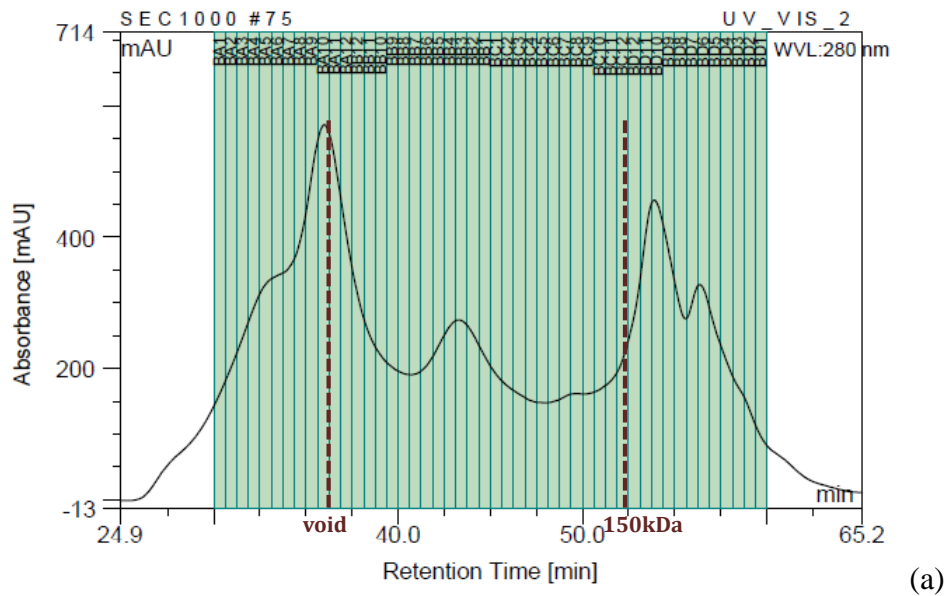
**Figure 4. 2. Spindly interacts with SAC components.**

HEK293 cells were transfected with YPF-SAC proteins (as indicated in the figure) and enriched in mitosis (Nocodazole 40ng/mL). Immunoprecipitation was performed with anti-Spindly antibody and samples were analysed by Western blot. Rabbit IgG were used as negative control for all the samples. Antibodies as indicated. Input represents the whole cell lysate. YPF-E: YFP-empty vector.

Bearing in mind that data in figure 4.2 were obtained under the conditions where SAC proteins were over-expressed, we decided to use size-exclusion chromatography to further study the association between Spindly and the SAC components at endogenous levels. To this end, HEK293 cells were mitotically enriched by double Thymidine block and release in Nocodazole for 12 hours. Thymidine inhibits DNA synthesis synchronising cells in S phase (Thomas and Lingwood, 1975), so then the release in Nocodazole allows cells to proceed to M phase where they will be halt again, since the lack of MTs will trigger SAC activation (Spurck *et al.*, 1986).

Fractionation was performed on a SEC-1000 column and fractions were probed for different SAC components by Western blot (Fig. 4.3).

Figure 4.3 reveals that Spindly and BubR1 partially co-elute (panel (b), C fractions (~ 250 kDa)) suggesting that these two proteins could be within the same complex.



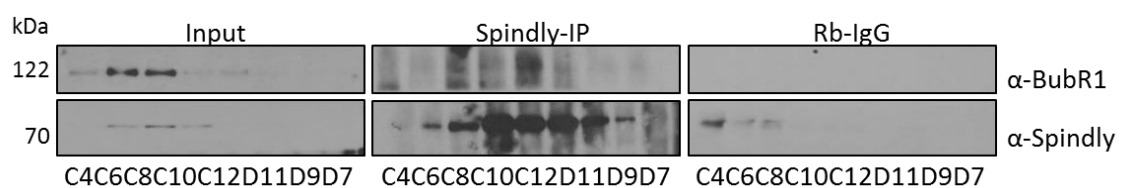
**Figure 4. 3. Spindly co-elutes with BubR1.**

HEK293 cell lysates mitotically enriched by double Thymidine (2mM) block and released in Nocodazole (40 ng/mL) for 12 hours, were fractionated through a SEC-1000 column. (a) Elution profile of the total cell lysate; (b) Western blot analysis for the indicated antibodies. Input represents the whole cell lysate.

Interestingly, Mad1 and Mad2 co-elute with ZW10, which indicates therefore a presence of two separate complexes containing SAC components at kinetochores (Fig. 4.3, panel (b), D fractions (~150 kDa)).

This is in line with data from other laboratories that have already identified the association of different proteins of the SAC with different kinetochore proteins: whereas Mad1/Mad2 associate with kinetochores thanks to the RZZ complex (Buffin *et al.*, 2005), BubR1-Bub3 bind through Bub1/Kn11 (Sharp-Baker and Chen, 2001).

To further validate this result we immunoprecipitated Spindly from C and D fractions (see Fig. 4.3) and confirmed the interaction with BubR1 (Fig. 4.4).



**Figure 4. 4. Spindly immunoprecipitates BubR1.**

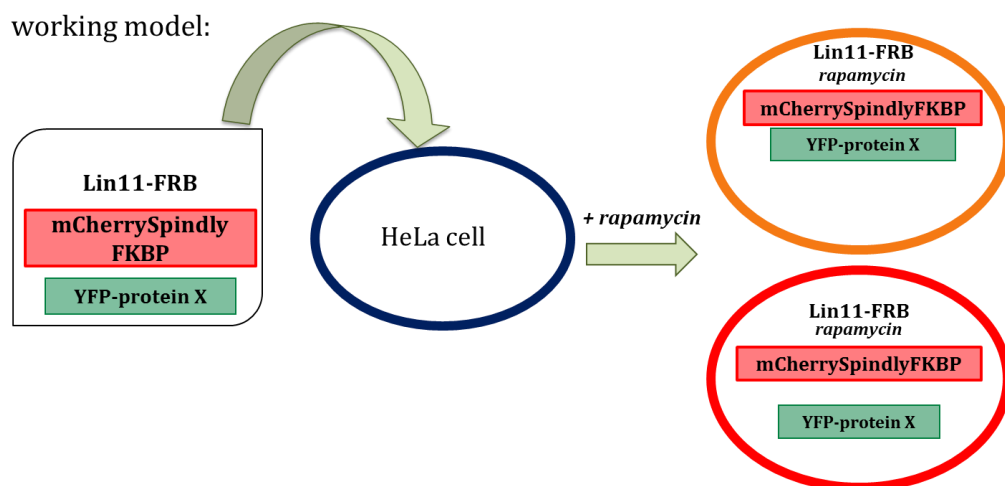
Immunoprecipitation of endogenous Spindly from SEC- fractions (Cs and Ds) was performed using anti-Spindly antibodies. 200 µl of the original fraction were used to immunoprecipitate Spindly. Rabbit IgG were used as negative control. Samples were analysed by Western blot with the indicated antibodies. Input represents the starting material.

Data collected from these assays support our hypothesis of a physical crosstalk between Spindly and the SAC, likely to be mediated via BubR1. In fact, although BubR1 is loaded onto kinetochores earlier (in prophase) it could be an adaptor partly responsible for targeting Spindly to or stabilising it onto kinetochores.

## 4.4 Spindly and SAC checkpoint proteins interact “*in vivo*”

Rapamycin is a small molecule that can induce dimerisation of the proteins FKBP12 (or FKBP) and mTOR (or mTOR’s minimal rapamycin binding fragment FRB). This dimerisation capacity has been established as a technique that allows controlling the association of two proteins (expressing FKBP and FRB tags) in living cells (Putyrski and Schultz, 2012).

To corroborate our previous results, we sought to analyse the interaction between Spindly and different SAC components “*in vivo*”, taking advantage of this method. We thus generated a mCherry-Spindly-FKBP tagged construct that will bind to any protein containing a FRB domain upon addition of rapamycin. We have chosen to use a Lin11-FRB to drive the mis-localisation of mCherry-Spindly-FKBP to the plasma membrane, so we will be able to screen for positive interaction upon co-localisation of mCherry and YFP proteins at the plasma membrane (Fig. 4.5).

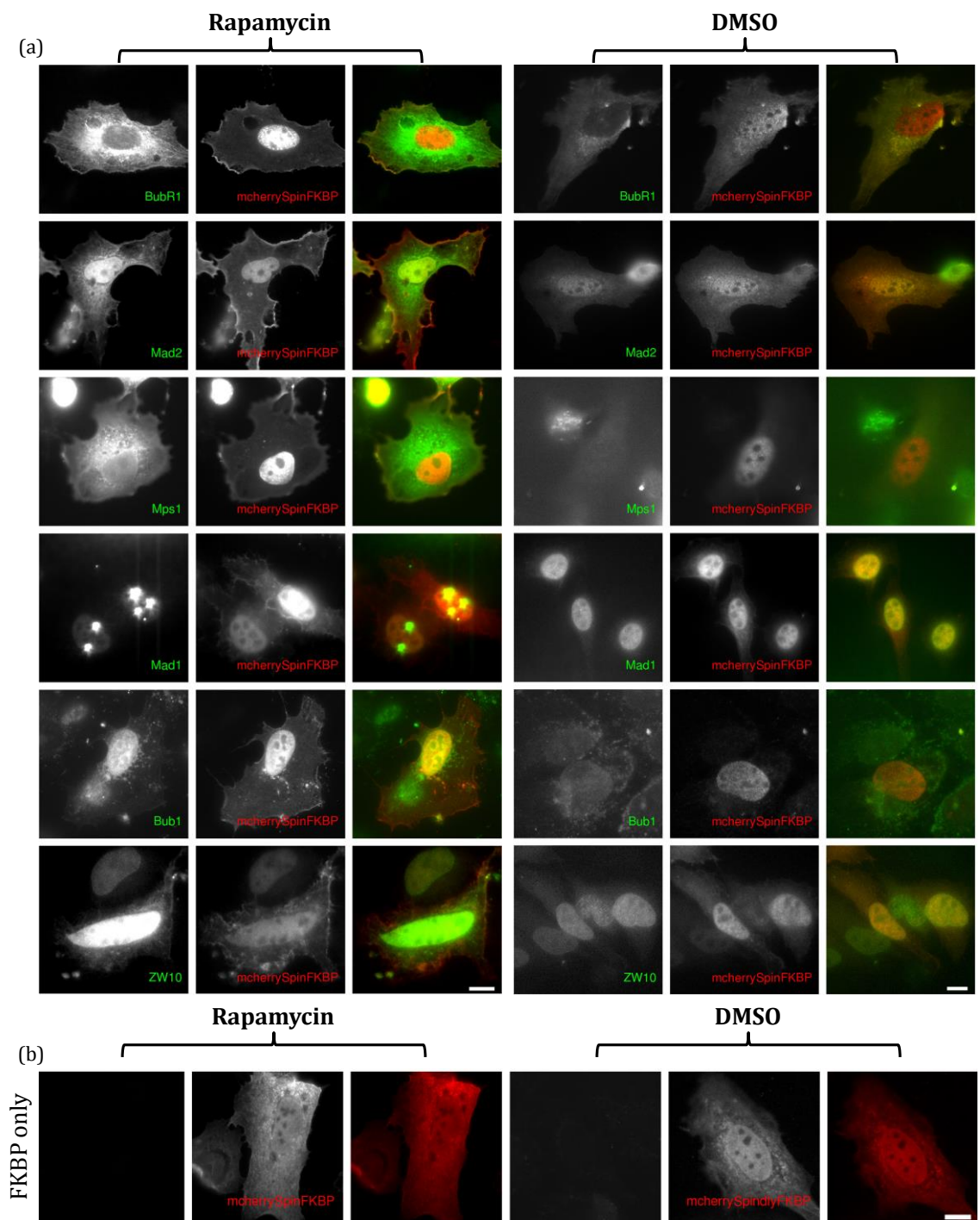


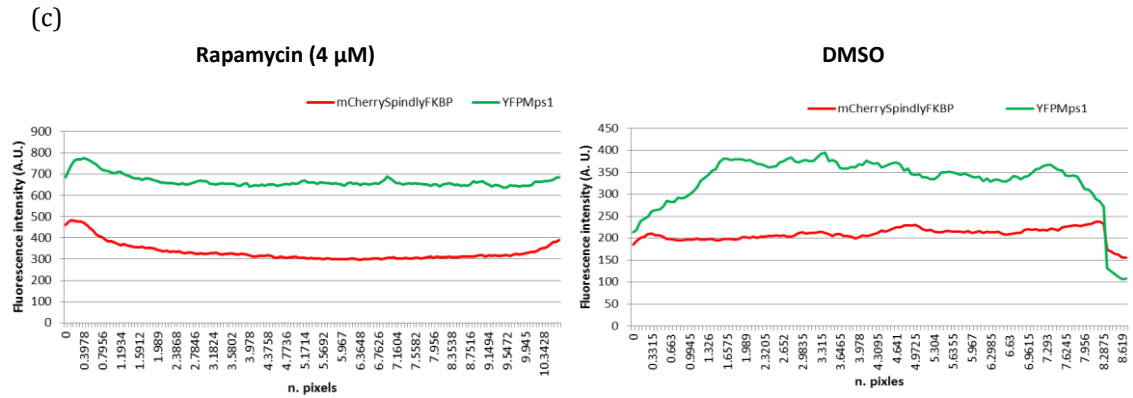
**Figure 4. 5. The FRB-FKBP system.**

Schematic representation of the work flow for cell transfection and subsequent rapamycin mis-localisation assay. Screening was carried out by looking for either red plasma membrane (= interaction does not occur) or orange plasma membrane (= interaction does occur).

We tri-transfected HeLa cells with the YFP tagged protein of interest (the different SAC effectors to test), Lin11-FRB and mCherry-Spindly-FKBP. After about 40 hours from the transfection, we treated cells with rapamycin (4  $\mu$ M for 1 hour at 37°C), then fixed with PFA and visualised protein localisation.

Figure 4.6 (a) demonstrates the mis-localisation of BubR1, Mad2 and Mps1 to the plasma membrane upon rapamycin administration (left-hand side panel). Conversely, Mad1 and Bub1 did not mis-localise to the plasma membrane upon treatment. Also, we observed only a weak interaction of Spindly with ZW10, suggesting that their association could be mitotic specific. Panel (b) in figure 4.6 indicates neither membrane localisation for the FKBP construct when transfected on its own nor background YFP-fluorescence.



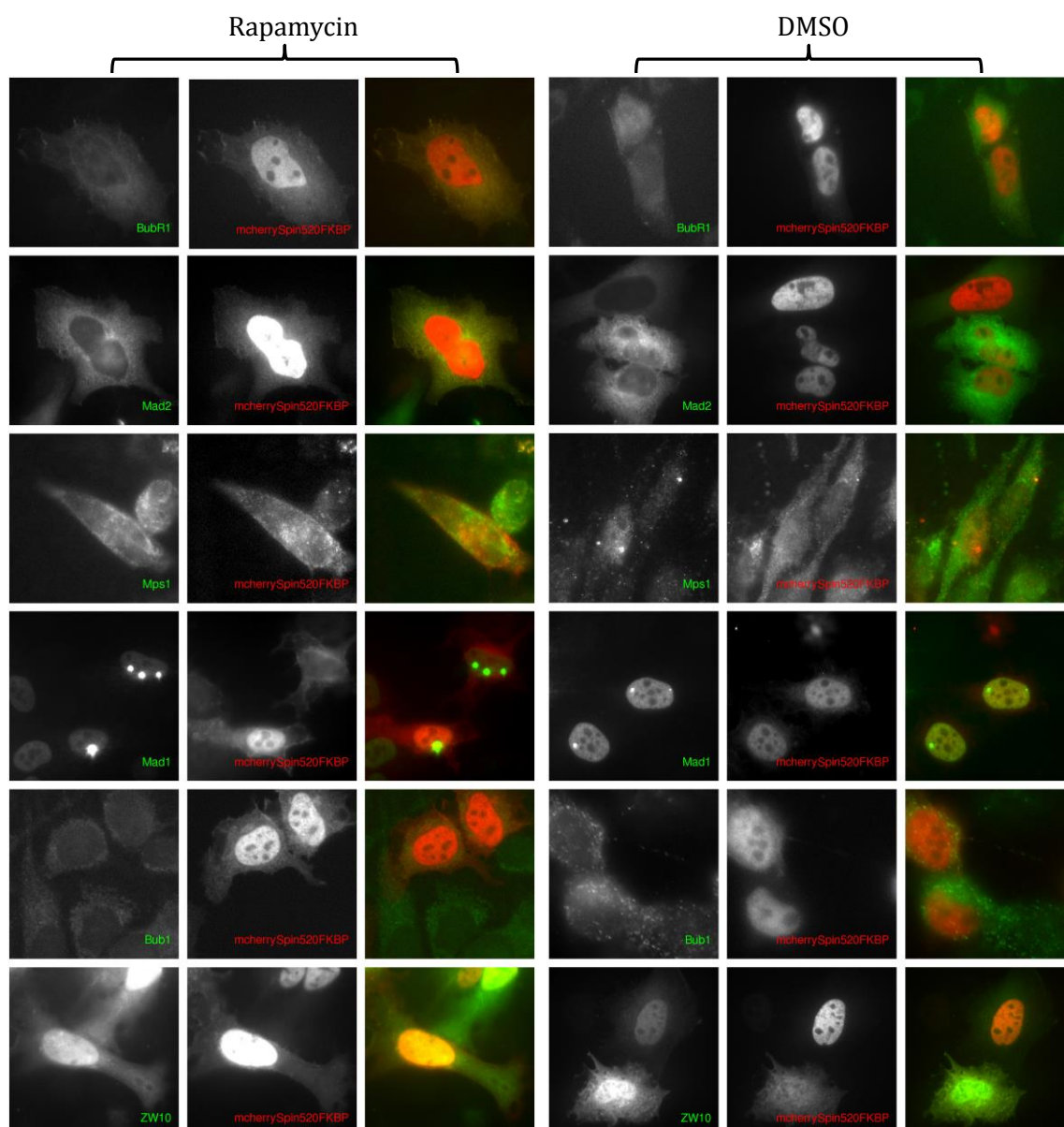


**Figure 4. 6. Mis-localisation of Spindly reveals interaction with SAC components.**

(a) HeLa cells were transfected with the indicated SAC component (YFP tagged), mCherrySpindly-FKBP and Lin11-FRB for 40 hrs. Rapamycin (4  $\mu$ M) was administered for 1 hour (at 37  $^{\circ}$ C) and then cells were visualised: SAC protein (green), Spindly (red). (b) Cells were transfected only with the FKBP construct and treated with rapamycin as negative control. Left-hand side panel Rapamycin treated samples; right-hand side DMSO treated samples. 60X magnification. Scale bar 10 $\mu$ m.

(c) Measurements conducted for Mps1 mis-localisation recruitment at the plasma membrane; values were obtained by drawing a line between the two edges of the cell (excluding carefully the nucleus, where often an over-expression was registered) and then the fluorescence intensity was measured using a plug-in in FiJi (multichannel plug-in) and plotted with Excel. Similar measurements were conducted for all the SAC components tested. For each experiment 50 cells were measured and then the average values for each channel were plotted.

It has been reported that the recruitment of Spindly at KT is dependent on the RZZ complex, since defects in ZW10 expression abrogate this process and also that the Spindly C-terminus is important for its KT localisation (Griffis *et al.*, 2007) (Gassmann *et al.*, 2008) (Barisic *et al.*, 2010). We therefore hypothesise that Spindly associates to KT via its C-terminus in relation with the RZZ complex. To validate this hypothesis, we generated a mCherrySpindly-FKBP construct truncated of the C-terminal region (1-520 amino acids) and repeated the rapamycin mis-localisation experiment (Fig. 4.7).



**Figure 4. 7. mCherry-Spindly520-FKBP can still interact with ZW10 and with the SAC components.**

HeLa cells were transfected with different YFP-tagged proteins, mCherry-Spindly520-FKBP and Lin11-FRB for 40 hrs. Rapamycin (4  $\mu$ M) was administered for 1 hour (at 37 C) and then cells were fixed and visualised: SAC protein (green), Spindly (red). Left-hand side panel Rapamycin-treated samples; right-hand side DMSO-treated samples. 60X magnification. Scale bar 10 $\mu$ m.

Figure 4.7 reveals that all the proteins tested are still able to associate with Spindly at the plasma membrane as in the WT experiment (Fig. 4.6), suggesting that the C-terminus is not the site required for binding or at least it is not the only site involved (Fig. 4.7) (for measurements analysis see graphs in **Chapter 8. Appendix- Fig. 8.2**).



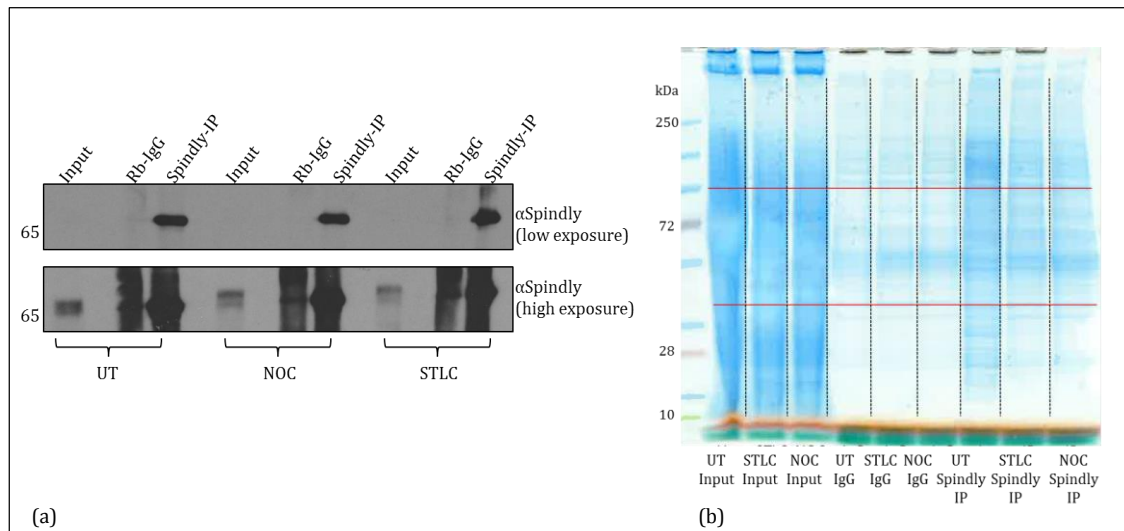
Previous reports have already revealed that Spindly possesses different sites for KT-binding within its structure besides those present in the *C*-terminus (Barisic *et al.*, 2010) (Moudgil *et al.*, 2015); our data therefore confirm that the *C*-terminus of Spindly is dispensable for association with kinetochore/SAC components.

## 4.5 Identification of novel binding partners for Spindly in mitosis

To identify novel mitotic proteins interacting with Spindly, it was decided to exploit the mass spectrometry (MS) technique to gain insights into the prometaphase complex of which Spindly could be a component.

HEK293 cells were mitotically enriched and harvested by mitotic shake-off upon overnight treatment with either STLC or Nocodazole. Cells without mitotic drug treatment were also collected as control. We used these two treatments with the aim to analyse two different statuses of KT-MT attachments that we think could give us different interactors of Spindly. Nocodazole is a drug that arrests cells in M phase; it inhibits MTs polymerisation and therefore the formation of the metaphase spindle, blocking the cells in prometaphase and leaving KTs unattached (Spurck *et al.*, 1986). On the other side, STLC is a potent inhibitor of kinesin Eg5 that arrests cells similarly in M phase, but in this case they are able to make a dense microtubule network since this drug does not affect microtubules polymerisation (Skoufias *et al.*, 2006). STLC treated cells halt in mitosis with a monopolar spindle; so in this case KT-MT attachments occur and therefore the interactions between proteins at KTs are different.

Cells were lysed and immunoprecipitation was performed by incubation with anti-Spindly or control Rabbit IgG antibodies overnight (Fig. 4.8, (a)). Subsequently, samples were fractionated by SDS-PAGE and the gels were stained. The lanes were split into three horizontal sections (as indicated from red lines in Fig. 4.8, (b)) and into eight vertical sections (as indicated from black lines in Fig. 4.8, (b)) and the gel pieces were processed for mass spectrometry analysis (see 2.9). In parallel, samples were run to confirm positive pull-down of endogenous Spindly from all the samples (Fig. 4.8 (a)).



**Figure 4. 8. Spindly-IP for mass spectrometry analysis.**

(a) HEK293 cells were enriched in mitosis and immunoprecipitation was performed with anti-Spindly antibody. Rabbit IgG was used as negative control. Samples were then blotted with indicated antibodies Input represents the whole cell lysate. (b) The immunoprecipitates were resolved on a SDS-polyacrylamide gel and stained with Instant Blue. Gels were divided into the indicated sections (black and red lines) and processed for mass spectrometry analysis. UT: untreated samples; NOC: nocodazole-treated samples; STLC: s-trityl-L-cysteine treated samples.

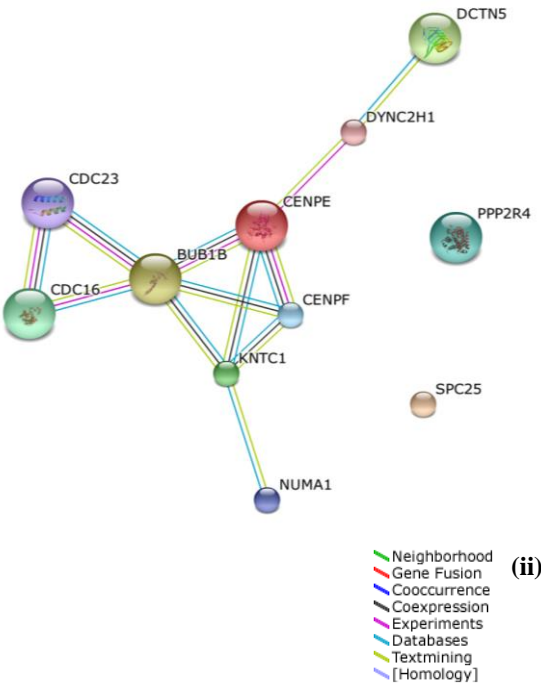
Analysis of the datasets obtained was conducted with the MaxQuant software (Cox and Mann, 2008), aimed to identify proteins enriched in the Spindly immunoprecipitation samples. This software allows us to run the .raw data against a contaminant database, to eliminate possible contaminants (like keratins) present in our datasets, as well as against a Human reverse database, to eliminate all the false positive hits (those that will align with this database will be discarded). After this initial analysis, we subsequently screened our datasets for mitotic and kinetochore enriched proteins that could be part of the ‘Spindly-mitotic complex’. Specifically we took into account the number of peptides identified for each protein and the intensity value at which they were identified. We compared the list obtained for the “Spindly IP” dataset with the “IgG” dataset (our negative control) and proceeded with the analysis only for those proteins that gave an intensity value equal to zero in the “IgG” dataset.

Figure 4.9 summarises the data from the mass spectrometry analysis. In the left hand-side ((i)) of panel 1 are tables highlighting all the mitotic related proteins enriched in the Spindly-IP datasets under different conditions: untreated cells (UT) (a), Nocodazole treated cells (NOC) (b), and STLC treated cells (c); in the right hand-side ((ii)) of panel 1 instead is represented a network analysis performed using the String-bd software (Jensen *et al.*, 2009) for all the conditions tested. This analysis allows to link proteins according to the information already available on physical and functional protein-protein interactions, proved either by experimental data or computationally prediction (see legend underneath each network analysis, Fig. 4.9, panel (1), (ii)). Panel 2 is a summary of the identified protein functions obtained from the UniProt Human Database.

(1)

KT BINDING PROTEINS	PEPTIDE	INTENSITY SpindlyIP	INTENSITY IgG
CENP-F	81	12206621	0
CENP-E	35	6929300	0
KNTC1 (ROD)	6	3418200	0
Spc25	3	1033200	0
SAC RELATED PROTEINS			
BUB1B	9	1931000	0
CDC23	10	3002890	0
CDC16	5	3429220	0
PHOSPHATASES			
PP2AB56	6	1171100	0
CENTROSOME ASSOCIATED PROTEINS			
NUMA1	12	2083500	0
DYNEIN/DYNACTIN			
DYNEIN 2 HEAVY CHAIN	4	1241200	0
DYNACTIN subunit 5 (p25)	1	568240	0

(i)

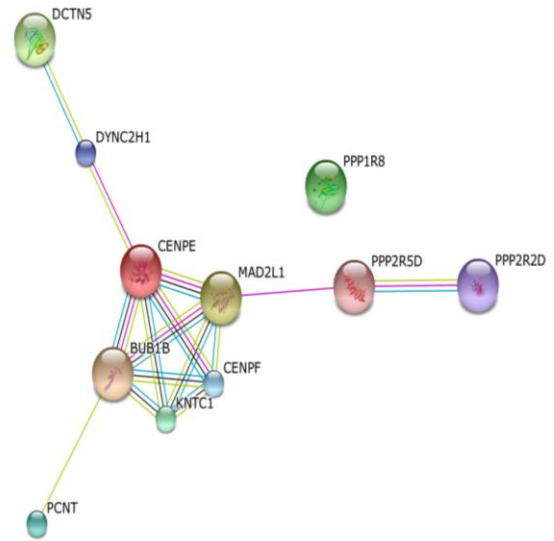


(ii)

(a)

<b>KT BINDING PROTEINS</b>	PEPTIDE	INTENSITY SpindlyIP	INTENSITY IgG
CENP-F	81	81830590	0
CENP-E	35	16080000	0
KNTC1 (ROD)	6	600330	0
<b>SAC RELATED PROTEINS</b>			
BUB1B	9	1185600	0
MAD2	11	411520	0
CDC16	5	407640	0
<b>PHOSPHATASES</b>			
PP2A B56 iso delta	6	524270	0
PP2A B55	3	126810	0
PP1 subunit 12	7	1393539	0
PP1 subunit 8	4	919300	0
<b>CENTROSOME ASSOCIATED PROTEINS</b>			
PERICENTRIN	36	15200000	0
<b>DYNEIN/DYNACTIN</b>			
DYNEIN 2 HEAVY CHAIN	4	1421100	0
DYNACTIN subunit 5 (p25)	1	52795	0

(i)

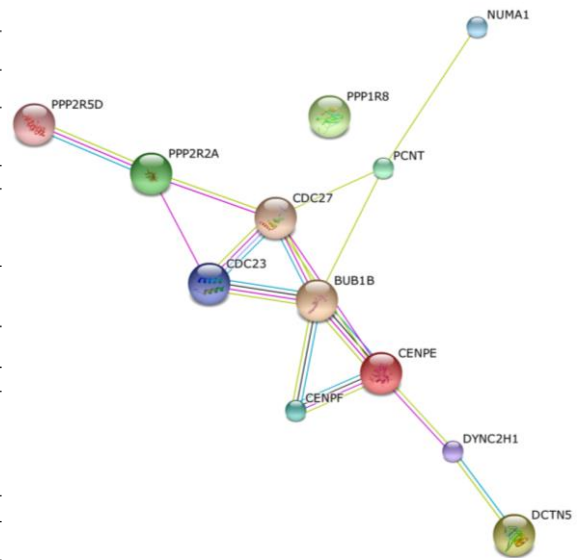


(ii)

(b)

<b>KT BINDING PROTEINS</b>	PEPTIDE	INTENSITY SpindlyIP	INTENSITY IgG
CENP-F	81	16028542	0
CENP-E	35	4829500	0
<b>SAC RELATED PROTEINS</b>			
BUB1B	9	1547660	0
CDC23	10	1700900	0
CDC27	10	2491600	0
<b>PHOSPHATASES</b>			
PP2A B56	6	299880	0
PP2A B55	6	381080	0
PP1 subunit 12	7	1036300	0
PP1 subunit 8	4	2551900	0
<b>CENTROSOME ASSOCIATED PROTEINS</b>			
PERICENTRIN	36	6738100	0
NUMA1	12	737414	0
<b>DYNEIN/DYNACTIN</b>			
DYNEIN LIGHT CHAIN	3	2190800	0
DYNEIN 2 HEAVY CHAIN	4	3356541	0
DYNACTIN subunit 5 (p25)	1	52795	0
<b>MITOTIC KINASES</b>			
Cyclin B1	7	1049800	0
PLK1	3	835290	0

(i)



(ii)

(c)

(2)

PROTEIN NAME	PROTEIN FUNCTION
CENP-E	centromere protein E. Essential for the maintenance of chromosomal stability through efficient stabilization of microtubule capture at kinetochores. Plays a key role in the movement of chromosomes toward the metaphase plate during mitosis.
CENP-F	centromere protein F (mitosin). Required for kinetochore function and chromosome segregation in mitosis. Required for kinetochore localization of dynein, LIS1, NDE1 and NDEL1.
BUB1B	Essential component of the mitotic checkpoint. Required for normal mitotic progression. Required also to monitor kinetochore motor CENP- E and its kinetochore localisation.
DCTN5	Subunit 5 of dynactin. It is a component of the pointed-end subcomplex and is thought to bind membranous cargo. (p25)
DLIC	Cytoplasmic dynein 1 light intermediate chain 1. Acts as one of several non-catalytic accessory components of the cytoplasmic dynein 1 complex.
DYNC2H1	dynein, cytoplasmic 2, heavy chain 1. Involved in the retrograde transport in the cilium.
KNTC1	kinetochore associated 1. Required for the assembly of the dynein-dynactin and MAD1-MAD2 complexes onto kinetochores.
SPC25	SPC25, NDC80 kinetochore complex component, homolog. Is required for chromosome segregation and spindle checkpoint activity.
PCTN	Pericentrin. Plays a role, together with DISC1, in the microtubule network formation. Is an integral component of the pericentriolar material (PCM).
MAD2L1	MAD2 mitotic arrest deficient-like 1. Required for the execution of the mitotic checkpoint which monitors the process of kinetochore- spindle attachment and inhibits the activity of the APC/C.
NUMA1	nuclear mitotic apparatus protein 1. Required for maintenance and establishment of the mitotic spindle poles.
PPP1R8	protein phosphatase 1, regulatory subunit 8; Inhibitor subunit of the major nuclear protein phosphatase-1 (PP-1).
PPP2R4D	protein phosphatase 2A activator, regulatory subunit 4.
PPP2R2D	protein phosphatase 2, regulatory subunit B, delta of protein phosphatase 2A (PP2A) that plays a key role in cell cycle by controlling mitosis entry and exit.
PPP2R5D	protein phosphatase 2, regulatory subunit B, delta.
CDC23	cell division cycle 23 homolog; component of the anaphase promoting complex/cyclosome (APC/C).
CDC27	cell division cycle 27 homolog.
CDC16	cell division cycle 16 homolog.
CYCLIN B1	G2/mitotic-specific cyclin B1. Essential for the control of the cell cycle at the G2/M transition.
PLK1	Serine/threonine-protein kinase that performs several important functions throughout M phase of the cell cycle.

**Figure 4. 9. Identification of novel Spindly binding partners in mitotic cells.**

**Panel 1:** (i) Table summarising molecular candidates enriched in Spindly IP samples and that scored zero for the intensity value in the IgG IP samples. (a) Untreated cells; (b) Nocodazole treated cells; (c) STLC treated cells. (ii) STRING network diagram representing the network for the Spindly-IP enriched proteins. This “evidence view” was obtained from String-bd.org, a database of known and predicted protein interactions derived from different sources. Colour legend is reported to explain the different connection lines. Single lines mean confidence of interaction, whereas double lines indicate evidence for interaction. Each protein is represented by a node. (a) Untreated cells; (b) nocodazole treated cells; (c) STLC treated cells.

**Panel 2:** Summary of the functions of enriched proteins (adapted from information obtained from Uniprot.org) .

The three sets of data gathered from the mass spectrometry analysis revealed some similarities among the proteins enriched in the three different conditions tested, with consistency in the intensity and peptide counting registered (Fig. 4.9): the kinetochore binding proteins CENP-E and CENP-F; the SAC component BubR1 (BUB1B); the phosphatase PP2A (various subunits) and the subunit 5 (p25) of dynactin. The presence of different subunits of dynactin in the MS data is a further confirmation of the association between Spindly and dynactin (as already proved in **Chapter 3**), no matter what phase the cell is in. Moreover, it is worth to point out that we also found other subunits of both dynein and dynactin in the MS data; these proteins were not included in our lists since their IgG values were not equal to zero. Similarly, the recurrence of BubR1 (BUB1B) in all the datasets strongly supports our former results about an association between Spindly and this SAC effector (see Fig. 4.4. and 4.6). Likewise, the presence of Rod (KNTC-1) corroborates our information, previously gained (see Fig. 4.1), about a connection between Spindly and the RZZ complex.

Interestingly, we could identify specific mitotic proteins in the mitotically synchronised samples: Mad1, a specific SAC component normally recruited at the beginning of mitosis, was highly enriched in both STLC and NOC treated samples, according to the fact that it is a specific mitotic kinetochore protein that does not present fast turnover KT-MT attachment (and consequently it gets retained at KTs upon STLC treatment). Conversely, Mad2 levels were higher in the NOC samples, in line with the fast turnover of this SAC component upon KT-MT attachment. In our data it was also recorded the presence of the SAC protein Bub3, which, as Mad2, was highly enriched upon NOC treatment. For all these interactors the IgG values were not equal to zero, so we did not proceed with further analysis. It is worth to point out as well that other mitotic kinetochore proteins were recorded at high levels of intensity in the mitotic samples (however also in this case IgG values were not equal to zero). We identified NudE and

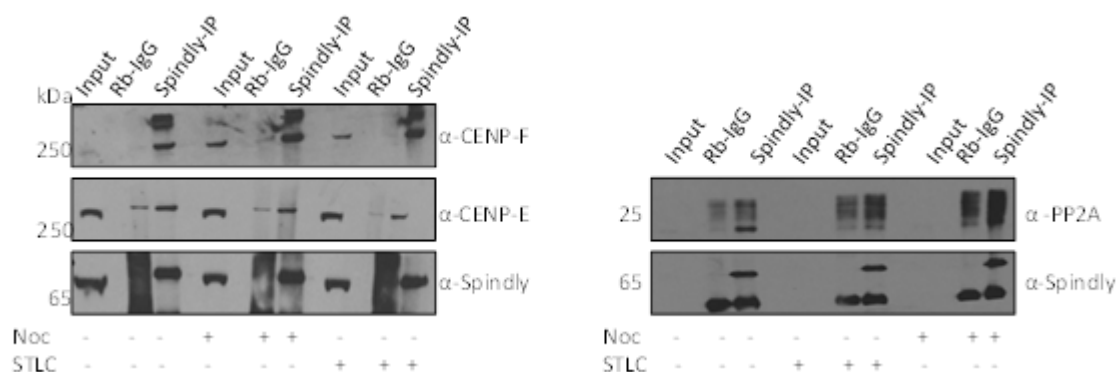
Lis1 in the UT data set as well as in the STLC data set (with intensity values, in the latter condition, definitely higher); these proteins have been reported as binding partner of dynein at KT's in mitosis, so it would be interesting in the future to further validate the data analysing the potential pathway of interaction that could involve Spindly and dynein at kinetochore in the mitotic division.

Looking at the whole set of data gathered from the mass spectrometry experiment (reported in **Chapter 8. Appendix- Paragraph 8.5**), it is possible to notice that, even though there were several kinetochore proteins highly enriched in the Spindly IP samples, this “category” of proteins was not the most abundant one. We did register the presence of cytoskeleton proteins, RNA factors, proteins involved in intracellular trafficking, actin binding proteins and centrosome proteins that could be new binding partners of Spindly. Since the initial aim of this study was not to study the whole interactome of Spindly but to identify specifically new mitotic binding partners in relation with Spindly-kinetochore localisation and functioning, we did not analyse the other potential interactors reported. However, it would be interesting to follow up this initial analysis and carry out a more comprehensive examination to identify Spindly's binding partner in different cellular contexts.

#### 4.5.1 Validation of Mass Spectrometry data

For some of the new kinetochore potential binding partners of Spindly identified in these analyses, we carried out further validation experiments to assess the interaction. HEK293 cells were cultured and treated as previously described for the MS analysis, and subsequently Spindly immunoprecipitation was performed (Fig. 4.10).





**Figure 4. 10. Spindly-IP validates the mass spectrometry results.**

HEK293 cells, either synchronised in mitosis (NOC or STLC) or untreated (UT), were harvested and 200/400 µg of the total protein was used to immunoprecipitate Spindly. Rabbit IgG was used as a negative control. Immunoprecipitated complexes were analysed by Western blot using the indicated antibodies. Inputs correspond to the whole cell extracts.

Results presented in figure 4.10 established the interaction between Spindly and the kinetochore proteins CENP-E and CENP-F, as well as the phosphatase PP2A. We noticed the presence of shifting bands in the blots for PP2A and CENP-F (Fig. 4.10); this could indicate post translational modifications for both proteins. The band presents in the CENP-F blot was particularly enriched after the pull-down, suggesting that it could bind to Spindly with high affinity; it could also be that it is not CENP-F related but it is something else that interacts with Spindly and it would need to be further investigated. PP2A has been demonstrated to undergo carboxy-methylation and phosphorylation (Kiely and Kiely, 2015), but the case reported in the above figure suggests that it could also potentially be subjected to ubiquitination. Surely this possibility would need to be deeply studied, but the previously proved high degree of regulation that is used to control this phosphatase (Kiely and Kiely, 2015), opens the possibility to different possible post translational modifications that have not been discovered yet.

## 4.6 Discussion

Proper interactions between kinetochores and spindle microtubules are crucial to ensure fidelity in chromosome segregation, and the mitotic checkpoint is required to monitor this process. The removal of checkpoint proteins from KT has to occur to promote the SAC silencing, and it has been shown to rely on the dynein/dynactin motor complex (Howell *et al.*, 2001). Together with the RZZ complex, Spindly is involved in the recruitment of the dynein/dynactin to KTs to allow the silencing process.

In this chapter we have shown an interaction between Spindly and the RZZ complex (see Fig. 4.1). Moreover, new associations between Spindly and the SAC components have been established. Spindly was demonstrated to associate with BubR1 and Mps1 when overexpressed (see Fig. 4.2 and Fig. 4.6). The association with BubR1 was further confirmed by SEC first and by co-immunoprecipitation of Spindly from fractions after. BubR1 was found to be the only SAC component tested that remains associated with Spindly throughout IP or SEC from cells arrested in early prometaphase (see Fig. 4.4) suggesting phase-specificity for this interaction. BubR1 is a component of the MCC, together with Bub3, Mad2 and Cdc20 (Sudakin *et al.*, 2001), and it associates with unattached/incorrectly attached kinetochores, playing an important role in kinetochore-microtubule interactions (Elowe, 2011). It gets localised to the kinetochore by binding to Bub1, which is recruited earlier by Mps1-mediated Knl1 phosphorylation (Vleugel *et al.*, 2013). BubR1 can therefore represent the link between Spindly and the SAC pathway. Moreover we corroborated the involvement of Spindly with the mitotic checkpoint “*in vivo*”, by using a system to mis-localise proteins at the plasma membrane and look for the specificity of the interaction (see Fig. 4.6). Exploiting the same system we additionally showed that the C-terminus of Spindly, although necessary for KT binding *in vivo*, is neither crucial for the interaction with the SAC components nor for that with the RZZ complex (see Fig. 4.7).

With the aim to detect novel mitotic binding partners of Spindly a mass spectrometry analysis was performed upon synchronisation of cells in mitosis, using different drugs to study different KT-MT attachment status. Kinetochore proteins behave differently in mitosis, associating to sites with different timing and showing different dynamics upon binding; indeed the pool of proteins that can be registered prior to MT attachment to KT is different from those present already right after their binding (see **Chapter 1- Paragraph 1.2.2.1**). Therefore, our goal with the mass spectrometry analysis was to identify binding partners of Spindly at the KT according to the diverse population of proteins present (prometaphase- no KT/MT attachment (NOC treatment) or prometaphase- KT/MT attachment (STLC treatment)). The wide approach of the mass spectrometry analysis allows in fact for a more comprehensive characterisation of the mitotic-interactome of Spindly in synchronised cells.

From the list of proteins obtained we identified new interesting Spindly interactors, such as CENP-E and CENP-F. During mitosis these CENP-E and CENP-F are kinetochore localised, but they possess different recruitment timing and they exert different functions. CENP-F is recruited from the nucleus at the beginning of mitosis and it is a stable kinetochore protein that stays until metaphase/anaphase transition (Zhu *et al.*, 1995). CENP-E, however, is recruited onto kinetochores in late prometaphase and is a ‘sensor’ factor for KT-MT attachments (Yao *et al.*, 2000). CENP-E is involved in checkpoint assembly: it interacts with BubR1 establishing a feedback signalling for activation/inactivation of the SAC (Mao *et al.*, 2003), which in turns promotes kinetochore localisation of Mad2 (Johnson *et al.*, 2004). Conversely, CENP-F has not been described as an active player of the spindle assembly checkpoint, but it directly binds to CENP-E promoting its kinetochore stabilisation (Chan *et al.*, 1998). Defects in CENP-E expression also affect kinetochore CENP-F localisation, indicating a reciprocal

control between these two proteins (Johnson *et al.*, 2004). Both these proteins were identified in our mass spectrometry analysis (see Fig. 4.9) as novel kinetochore binding partners of Spindly, and for both of them we could validated the interaction by immunoprecipitation (see Fig. 4.10). We therefore think that CENP-F could represent the stable kinetochore binding partner of Spindly, to which it could anchor from the beginning of prometaphase. Recent publications have demonstrated that Spindly has to undergo farnesylation at its C-terminus to get recruited to KT's (Holland *et al.*, 2015) (Moudgil *et al.*, 2015); both CENP-F and CENP-E have been shown to be farnesylated in mitosis (Ashar *et al.*, 2000). So, Spindly immunoprecipitations after treatment with farnesyl-transferase inhibitors should be repeated to confirm that the interactions seen in our results are specific and not due only to the presence of the farnesyl group.

Curiously, Spindly cannot interact with kinetochores in the absence of RZZ, which suggests that RZZ is essential to promote the interaction between Spindly and CENP-E and/or CENP-F. Additionally, since CENP-E is a known binding partner of BubR1, it is possible that the Spindly-BubR1 interaction is mediated by CENP-E. Further experiments will be needed to better understand the associations between Spindly and CENP-E and/or CENP-F. However the massive size of CENP-E and CENP-F and a dearth of full-length cDNAs complicate dissecting these interactions.

Generally, it is possible to separate kinetochore proteins into two large groups according to their kinetochore dynamics: (1) those that stably interact with unattached kinetochores and (2) those that transiently associate. Spindle checkpoint proteins show a rapid capacity of recovery at kinetochores after photo-bleaching (Mps1, Bub3, Cdc20 and Mad2), while the constitutive proteins (such as CENPs and KMN components) do not (Howell *et al.*, 2004) (Vink *et al.*, 2006) (Hori *et al.*, 2003). For example, BubR1, that is constantly incorporated into the MCC and released into the cytosol, turns over

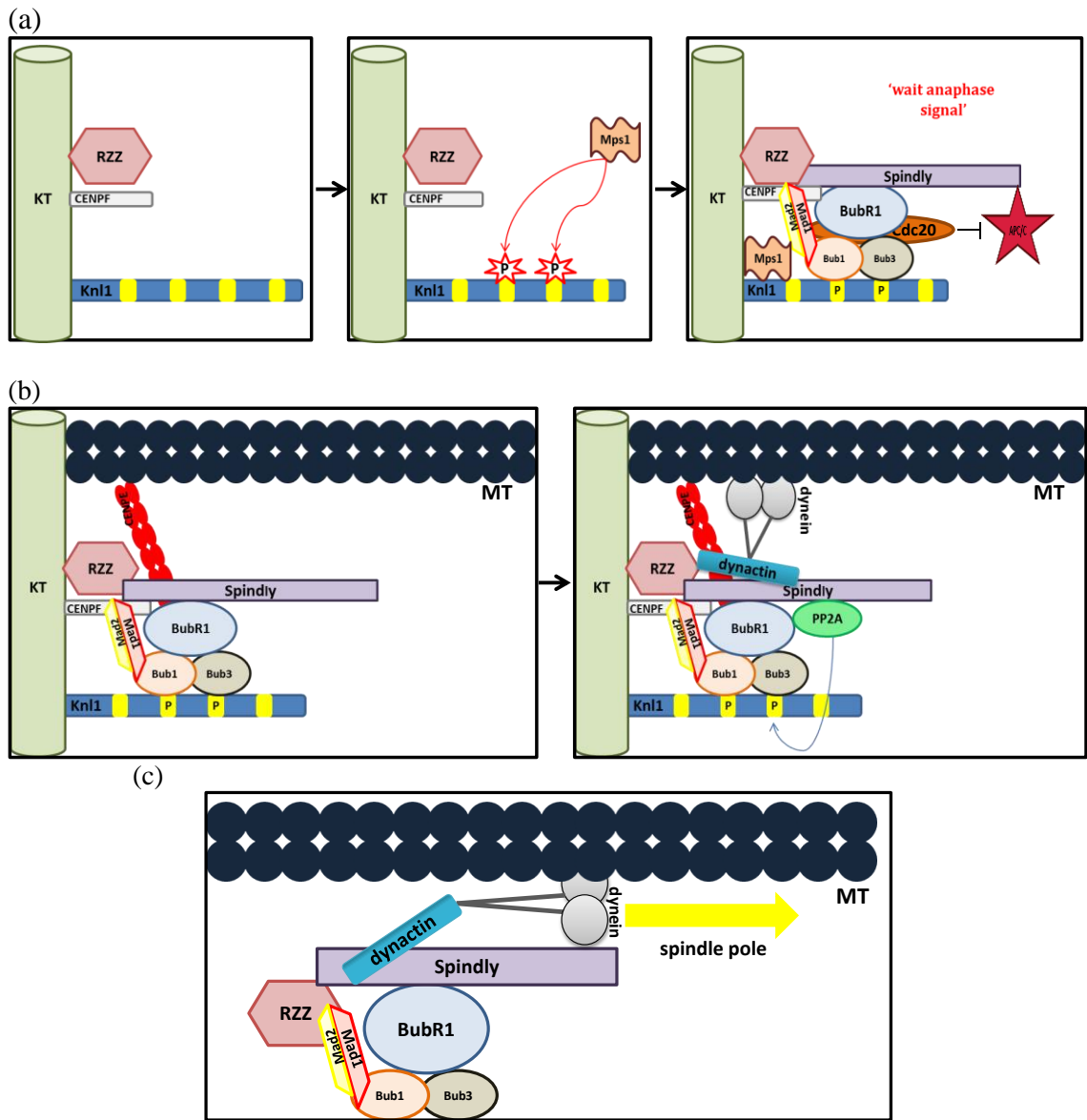
rapidly; conversely, Bub1, acting as a recruitment platform, is firmly associated with KTs (Howell *et al.*, 2004). When we tested GFP-Spindly for fluorescence recovery after photo-bleaching (FRAP) we registered a quite slow turnover and a very high immobile fraction of this protein (preliminary data, not shown). Publications have revealed that GFP-tagged RZZ components are not dynamic either (Famulski *et al.*, 2008), but recover slowly in FRAP experiments, similar to components of the KMN complex indicating that this complex is stably bound to prophase kinetochore (Hori *et al.*, 2003). Worth noticing is that in Spindly depleted cells the turnover of ZW10 was even further reduced (Barisic *et al.*, 2010), suggesting that Spindly promotes the turnover of the RZZ complex on KTs. The RZZ complex binds to kinetochores via Zwint, an outer plate kinetochore protein that ensures proper localisation of the complex in mitosis (Starr *et al.*, 2000). Similarly Spindly, by binding to CENP-F, could be stably tethered to kinetochores until MTs attach and dynein drives its dissociation.

In our MS data, we also found the phosphatase protein 2A (various subunits, see Fig. 4.9), recently identified as an essential player in the checkpoint silencing process (Nijenhuis *et al.*, 2014). Indeed, once the checkpoint has been satisfied, its effectors have not only to be removed, but also different substrates have to be de-phosphorylated to prevent re-/continued activation. Mps1-mediated phosphorylation of Knl1 promotes BubR1 recruitment (through Bub1), which, in turn, recruits PP2A to antagonise the action of the kinases that provide the signal to recruit SAC proteins (Espert *et al.*, 2014) (Nijenhuis *et al.*, 2014).

Here we observed the presence of an interaction between Spindly and PP2A, both of which are potentially playing a role in the silencing process: Spindly, by recruiting the dynein/dynactin motor complex and therefore promoting the stripping process, and PP2A, by de-phosphorylating Knl1 and therefore promoting the dissociation of the Bub1 and BubR1. The stripping and de-phosphorylation processes could happen

concurrently so to synchronise the SAC signalling at each kinetochore. Interestingly, in our MS data we found also protein phosphatase 1 (PP1) being pulled down with Spindly. Recently a PP2A and PP1 activation loop essential for faithful cell division was identified (Grallert *et al.*, 2015). Therefore, it would be interesting to understand whether Spindly could have a part in the regulation of this system.

In the scheme in Fig. 4.11, we propose a model for the functioning of Spindly in the mitotic checkpoint signalling. When mitosis starts, unattached kinetochores assemble hierarchically; transient kinetochore proteins get recruited and the SAC gets assembled activating the ‘wait anaphase signal’ (Fig. 4.11, (a)). Spindly is recruited as well and it binds to CENP-F, the RZZ complex and BubR1 (Fig. 4.11, (a), right hand-side). Attachment between microtubules and kinetochores occurs (Fig. 4.11, (b), left hand-side) and the checkpoint signalling senses the tension generated. Now the “wait anaphase signal” can be silenced. Therefore phosphatases become active and the dynein/dynactin motor complex is recruited to KTs (Fig. 4.11, (b), right hand-side). In turn, SAC components (as well as Spindly and the RZZ complex) are loaded and stripped away thanks to the motor complex dynein/dynactin (Fig. 4.11, (c)). Anaphase can now begin.



**Figure 4. 11. Proposed model for checkpoint assembly in mitosis.**

When a cell enters mitosis, kinetochore proteins are recruited to allow proper functioning throughout the process. In the ‘unattached kinetochore’ state (a), the spindle assembly checkpoint assembles to prevent anaphase onset. Mps1 phosphorylates Knl1 (a, middle panel) and allows the subsequent recruitment of Bub1, Bub3 and BubR1. Bub1 drives the localisation of Mad1 and Mad2, in cooperation with the RZZ complex. Spindly gets recruited as well (a, right-hand side panel). In the ‘attached kinetochore’ situation (b), CENP-E get localised via BubR1 (left-hand side panel) and the checkpoint can be silenced: PP2A dephosphorylates Knl1 allowing the release of Bub1, Bub3 and BubR1 and Spindly promotes binding of the motor complex dynein/dynactin to the SAC proteins (right-hand side panel). Finally, for anaphase to start, SAC components, Spindly and RZZ are stripped towards spindle poles (c).

Previous data showed that maintaining Spindly on KTs keeps the SAC active, whereas the lack of Spindly on KTs allows SAC silencing without the need to recruit dynein, but the mechanism by which the kinetochore presence of Spindly persistently activates the SAC has so far been unclear (Gassmann *et al.*, 2010). Our data that show interactions with two major SAC effectors (BubR1 and PP2A) allow us to form three hypotheses for the behaviour of Spindly: 1) Spindly could be creating aberrant phosphatase signalling that could affect the regulation of the phospho-equilibrium at KTs important for SAC activation/inactivation; 2) Spindly interaction with BubR1 could be critical to allow the silencing, thus it has to be removed to progress through mitosis; 3) Spindly presence on KTs could be sufficient for maintaining SAC activity retaining proteins at site.

Experiments to test these hypotheses will be essential for unravelling the real role of Spindly in SAC signalling.



## **5 . A new role for human Spindly in interphase**

## 5.1 Introduction

Spindly was identified through two RNAi screens in *Drosophila melanogaster* S2 cells in which mitotic and interphase phenotypes were analysed. In interphase cells, Spindly depletion generated alterations in cytoskeletal architecture with spiky and elongated microtubule-rich projections in contrast to the normal smooth rounded S2 cells. Moreover, expression of GFP-Spindly in live cells produced a pattern of continuously moving punctae of the protein, similar to how a microtubule plus-end binding protein would behave (Griffis *et al.*, 2007). Indeed, Spindly was shown to localise at the plus-tips of microtubules, when expressed at low levels together with EB1, a microtubule-associated protein present at growing ends in interphase cells (Griffis *et al.*, 2007) (Morrison *et al.*, 1998).

After the initial study in 2007, all of the subsequent publications on Spindly have been focused on describing its role during mitosis in human cells and worms (Gassmann *et al.*, 2008) (Yamamoto *et al.*, 2008) (Gassmann *et al.*, 2010) (Barisic *et al.*, 2010) (Cheerambathur *et al.*, 2013); thus there is no evidence on whether Spindly in other organisms plays a role similar to the one described for Spindly in interphase cells. We therefore sought to study the role of human Spindly in interphase, with a close look at microtubule localisation and cytoskeleton function.

It was previously demonstrated that the minus-end motor protein dynein is targeted to the microtubule growing ends via the p150 subunit of dynactin, which is itself recruited by EB1 and CLIP-170, proteins that bind to the plus-end of microtubules and regulate their dynamics (Folker *et al.*, 2005) (Duellberg *et al.*, 2014). This cytoplasmic motor complex dynein/dynactin was also described to be involved in cytoskeleton reorganisation upon wounding and, more precisely, in directed cell movement (Palazzo *et al.*, 2001) (Faulkner *et al.*, 2000) (Smith *et al.*, 2000). Furthermore, dynein was

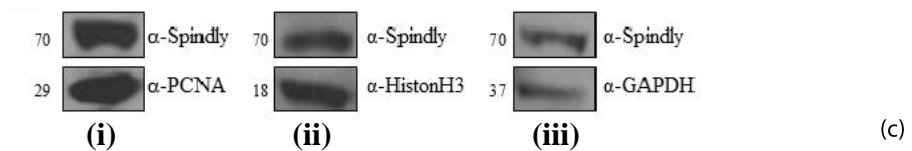
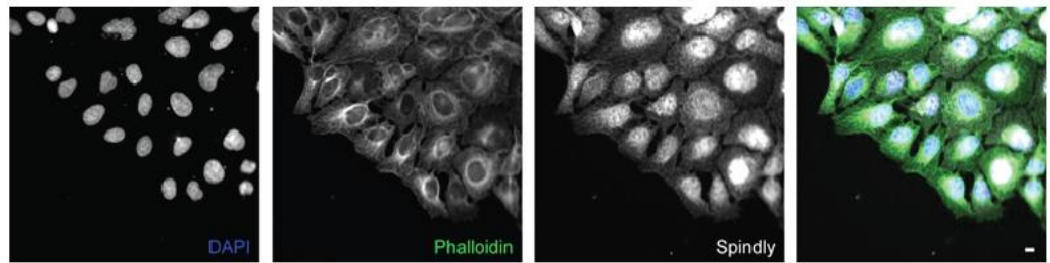
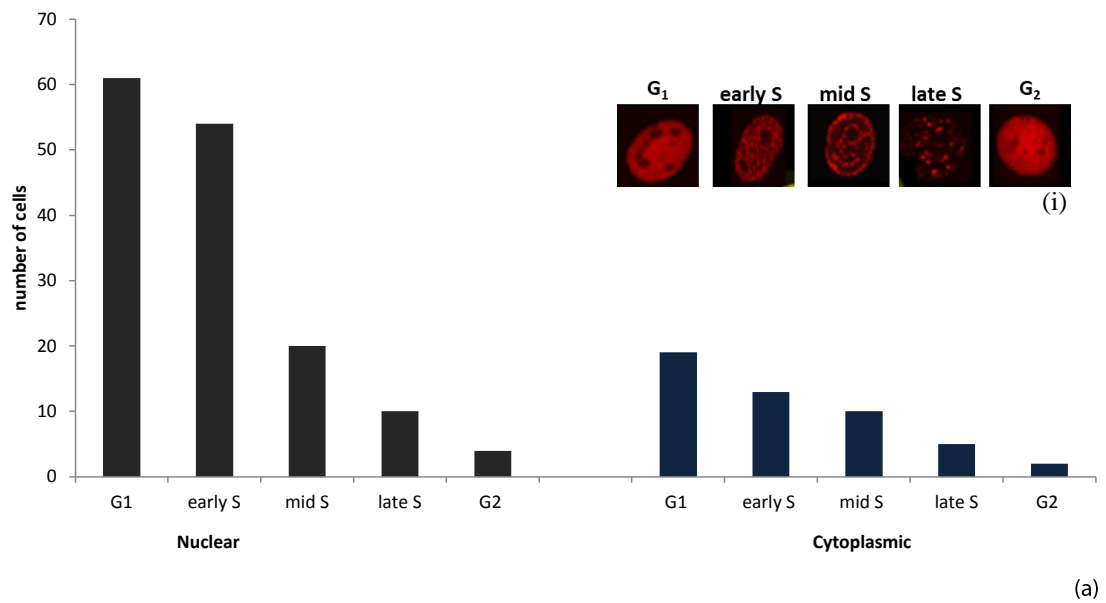
recorded helping neural progenitor cells to migrate from the ventricular zone during early brain development via an interaction with Lis1 (Reiner et al., 1993).

Given our previous results underlying the role of human Spindly as a dynein/dynactin molecular adaptor in mitotic cells (see **Chapter 3**), we hypothesised a similar function for Spindly in interphase cells.

In this chapter an important new function for Spindly in cell migration is described. We identified a direct role of human Spindly in wound healing and cell movement, with its specific localisation at focal adhesion tips upon wounding. These results delineate for the first time an interphase role for Spindly, corroborating our initial idea that places Spindly as a new adaptor for the dynein/dynactin motor complex in multiple cellular processes and in different cell cycle phases.

## 5.2 Localisation of human Spindly in non-mitotic cells

To date, there has been no data on human Spindly in non-mitotic cells and nothing is known about its localisation and function outside mitosis. To shed the light on this topic, we started analysing its distribution throughout the cell cycle using an antibody against the proliferating cell nuclear antigen (PCNA) as a cell cycle marker. PCNA is involved in DNA replication, clamping the DNA polymerase  $\delta$  in S phase; this allows visualising changes that occur during cell cycle on the genome duplicating machinery and therefore identify the different cell cycle stages (Easwaran *et al.*, 2005). Co-staining of U2OS cells with anti-Spindly and anti-PCNA antibodies has revealed that, even though Spindly is mainly a nuclear protein, is possible to identify a pool within the cytoplasm (Fig. 5.1, (a) and (b)). However there was no difference in the amount of Spindly seen in the cytoplasm regardless of the cell cycle stage as revealed by PCNA distribution (Fig. 5.1, (a)). Furthermore, fractionation of cell lysates and analysis of the different fractions (nuclear, cytoplasmic and chromatin) by Western blot has demonstrated the presence of Spindly not only in the expected nuclear fraction (Fig. 5.1, (c), (i)) but also in the chromatin (Fig. 5.1, (c), (ii)) and in the cytoplasmic ones (Fig. 5.1, (c) (iii)). The discovery of Spindly in these two other cell compartments indicates that Spindly might play additional functions besides its role in mitosis.



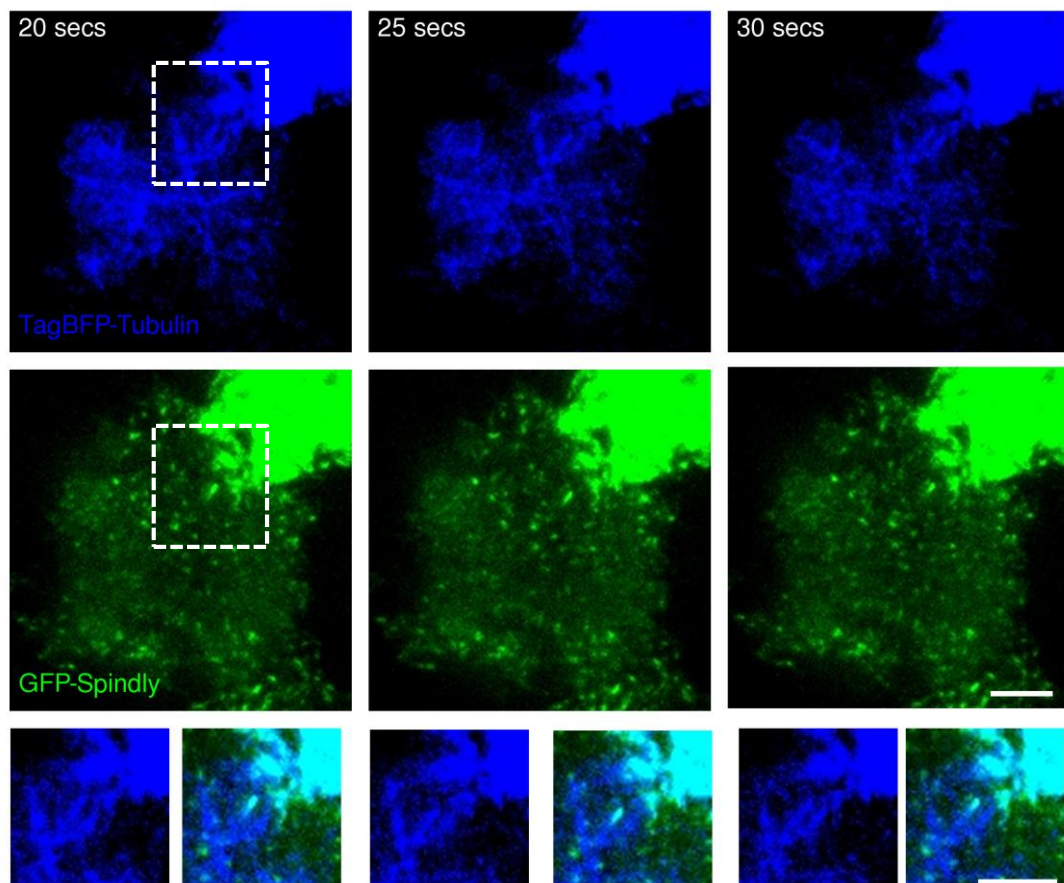
### Figure 5. 1. Spindly is not exclusively a nuclear protein.

Examination of Spindly localisation within interphase cells. (a) Analysis of Spindly distribution profile throughout the cell cycle using the PCNA cell cycle marker; cells in different phases were manually counted using as mask the characteristic PCNA staining reported in (i) (image adopted from Cardoso-lab.org) and then plotted using Excel. (b) Immunostaining of U2OS cells showing nuclei (DAPI, blue), actin (Phalloidin, green) and Spindly (white). 40X magnification. Scale bar 10µm. (c) Fractionation experiment of U2OS cell lysate (i) nuclear fraction, (ii) chromatin fraction and (iii) cytoplasmic fraction. Cross check analysis of the fractions was performed and results are reported in the appendix (see **Chapter 8- Fig. 8.3**).

To explore further the role of Spindly in interphase we asked whether the localisation seen in S2 cells was conserved in human cells. To this end we co-transfected U2OS cells with GFP-Spindly and BFP-Tubulin to study the potential association of Spindly with microtubules.

A live imaging experiment was carried out using total internal reflection fluorescence (TIRF) microscopy, a technique that allows better imaging of microtubules present at the basal cortex of the cell (Toomre and Manstein, 2001). Imaging was performed by Dr. Eric Griffis (University of Dundee).

Figure 5.2 shows a microtubule plus-end localisation for GFP-Spindly, which stays associated during a shrinkage and growth event.



**Figure 5. 2. Spindly localises at microtubule tips.**

Still images from TIRF time-lapse analysis of U2OS cells transiently co-expressing GFP-Spindly (green) and BFP-Tubulin (blue). Scale bar 5  $\mu\text{m}$ . Bottom of the panel shows magnified images derived from the boxed areas (dashed white box) followed over the time for both channels (green, Spindly; blue, Tubulin). Imaging performed by Dr. E. Griffis (University of Dundee).

The visualisation of human Spindly at microtubule tips together with the identification of new pools of Spindly in interphase cells bring up the possibility of a novel function for this protein in relation to the microtubule cytoskeleton, a function different from the already described mitotic one.

### 5.3 Human Spindly depletion affects cell migration

Given previous data that described Spindly in the cytoplasm of interphase cells (Fig. 5.1) and its presence at microtubules ends (Fig. 5.2), we sought to describe what cellular process (/es) Spindly might be involved with in non-mitotic cells.

It has already been demonstrated that microtubules are directly involved in cell migration and in the organization of cell polarity; many microtubule-associated proteins localising at their growing ends play parts in this process as well as the centrosome, the microtubule nucleating structure that directs the polymerisation of tubulin subunits (Schuyler and Pellman, 2001). The centrosome has to be repositioned between the leading edge and the nucleus upon wounding in order to set the direction of cell migration a critical step to allow wound closure (Palazzo *et al.*, 2001) (Magdalena *et al.*, 2003). Inhibition or overexpression of dynein and/or dynactin has been shown to disturb centrosome reorientation, but not to interfere with microtubule stabilisation (Palazzo *et al.*, 2001). Also, a separate pool of cytoplasmic dynein associating with actin-rich cortical cytoskeleton has been identified, together with its regulatory proteins dynactin and Lis1 (Faulkner *et al.*, 2000) (Dujardin *et al.*, 2003). It was proposed that, upon binding to microtubules, cortical dynein is involved in the generation of pulling forces, which in turns influences actin-based motility.

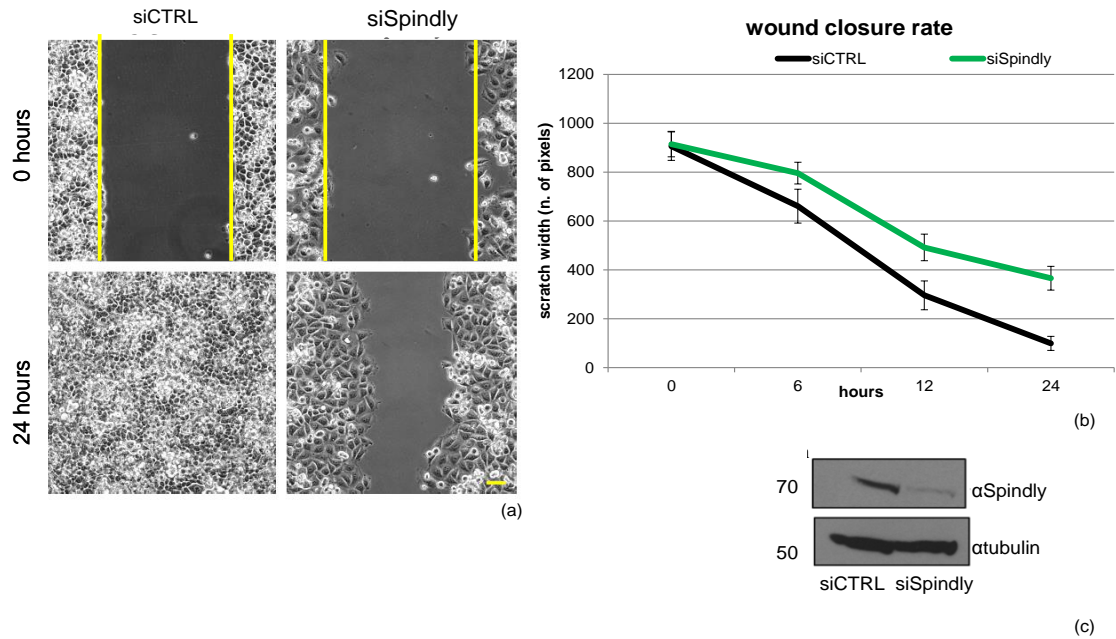
Localisation of Spindly at microtubule tips and its close interaction with the dynein/dynactin motor complex (see **Chapter 3**), led us to hypothesise that Spindly could also be involved in cell movement and in cytoskeleton re-organisation upon cells receiving the correct stimulus to begin directed movement. Thus, we carried out a scratch assay to study cell migration *in vitro* in Spindly-depleted cells in order to verify our hypothesis. This method is based on the generation of an artificial gap (“scratch”) on a monolayer of cells that will lead to movement of the cells on the edge toward the opening until contacting cells on the other edge, thus to close the “scratch”.



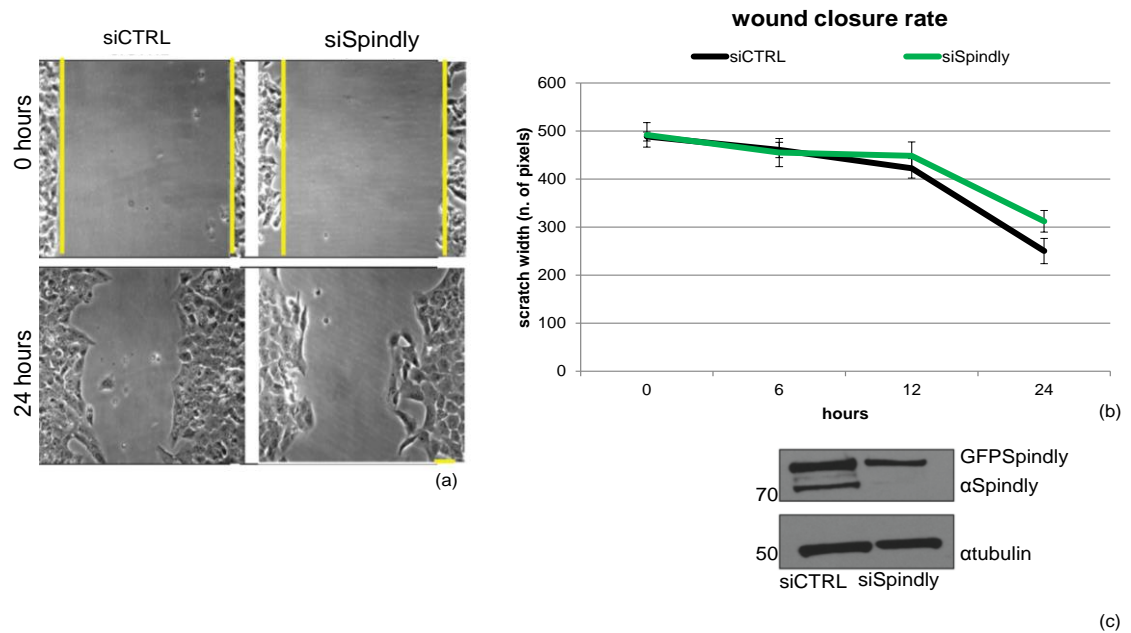
We silenced Spindly expression in U2OS cells by treatment with specific siRNAs for 96 hours and then seeded cells into two small wells separated by a silicon wall (IBIDI silicon inserts). Once confluence was reached, the insert was removed automatically generating a gap in the monolayer of cells thanks to the silicon wall. We followed the movement of the cells on the edge by live imaging for at least 24 hours and then analysed the movies by measuring the width of “scratch” over time. Cells depleted of Spindly showed slow closure rate with incapacity to close the gap after 24 hours; we recorded a much slower rate of cell migration into the “scratch” when compared with control cells (Figure 5.3 (panel 1)). Measurements were conducted by drawing lines from one edge toward the other (several measurements were performed for each image collected drawing lines from the top to the bottom of the “scratch”) and then values were averaged for each time point analysed. Results were further validated by scratching the monolayer of cells with a 200 $\mu$ L tip so to corroborate the data using a different technique and measurements were conducted in the same way (data not shown).

To confirm that this phenotype was strictly due to lack of Spindly expression, we then depleted the endogenous Spindly from U2OS cells expressing siRNA-resistant GFP-Spindly upon administration of doxycycline and performed the scratch assay again. Figure 5.3 (panel 2) shows that re-expressing an exogenous copy of Spindly rescued the migration phenotype; cells expressing GFP-Spindly showed a rate of closure similar to control cells.

(1)



(2)

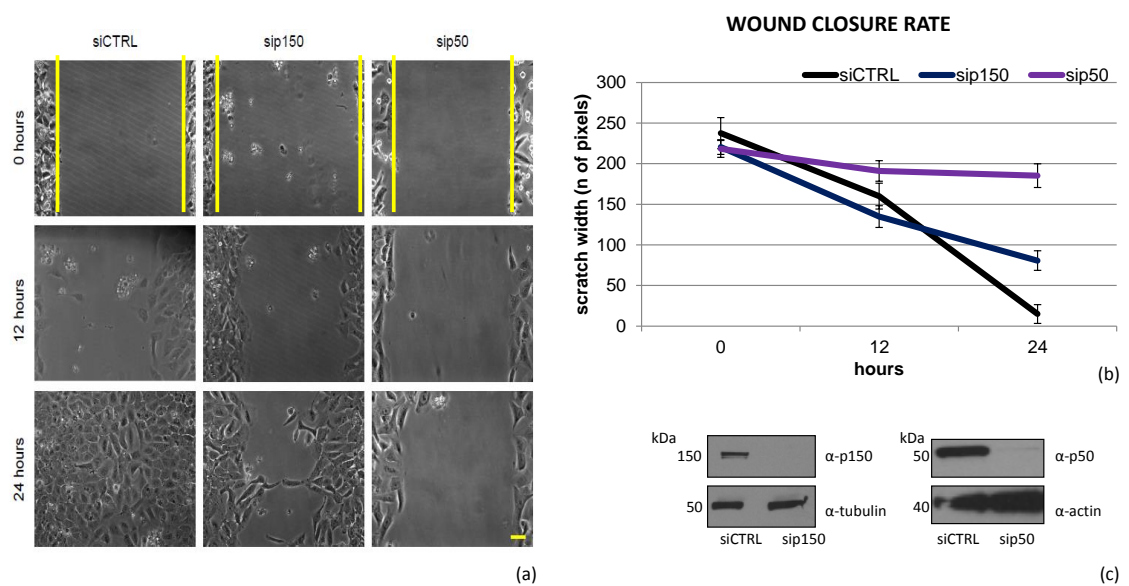


**Figure 5.3. Spindly plays a direct role in cell migration.**

**(1) Silencing of Spindly specifically affects cell migration.** (a) Still images from time-lapse analysis showing a delay in cell movement in U2OS Spindly depleted cells compared to control cells. Scale bar 50  $\mu$ m. (b) Quantification of the width of the scratch in control and Spindly depleted U2OS cells over time. Data indicate the mean  $\pm$  standard deviation (represented by error bars) from at least 3 independent experiments. (c) Western blot of same population of cells confirms the silencing; Tubulin was used as loading control.

**(2) Expression of GFP-Spindly rescues migration defects.** (a) Still images from time-lapse analysis showing rescued cell movement in U2OS GFP-Spindly depleted cells compared to U2OS GFP-Spindly control cells. Scale bar 50  $\mu$ m. (b) Quantification of the width of the scratch in U2OS GFP-Spindly control and depleted cells over time. Data indicate the mean  $\pm$  standard deviation (error bars) from at least 3 independent experiments (c) Western blotting of same population of cells confirms the silencing and the GFP-Spindly expression; Tubulin was used as loading control. [This figure represents experiments conducted with commercial inserts; anyway similar results were obtained scratching with a 200 $\mu$ L tip (data not shown)].

In parallel, we analysed U2OS cells depleted of dynactin subunits, either p150 or p50, and compared them with control cells. Dynactin has been observed at microtubules growing ends and at the cell cortex where it has been proposed to exert pulling forces on microtubules to promote cell migration (Busson *et al.*, 1998) (Vaughan *et al.*, 1999). We observed that dynactin depleted cells presented much slower migration rate compared to control cells (Fig. 5.4). The phenotype documented was comparable to the one described for Spindly depleted cells (see Fig. 5.3, (panel 1)).

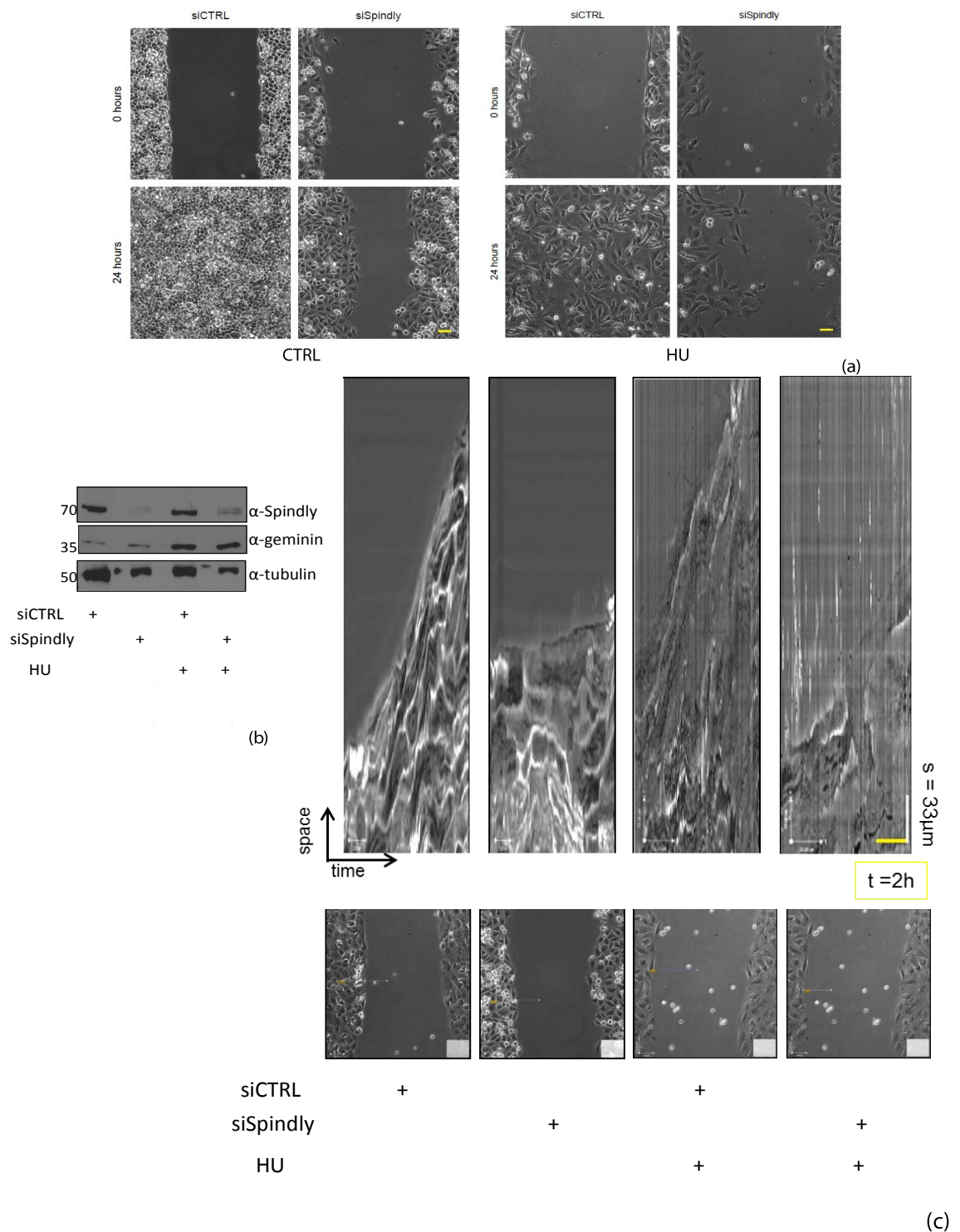


**Figure 5. 4. Depletion of dynactin prevents cell movement.**

(a) Still images from time-lapse analysis showing delayed cell movement in U2OS cells lacking dynactin (p150 or p50) subunits. 20X magnification. Scale bar 50  $\mu$ m. (b) Quantification of the width of the scratch in control and dynactin depleted U2OS cells over time. Data indicate the mean  $\pm$  standard deviation (represented by error bars) from at least 3 independent experiments. (c) Western blot of same population of cells confirms the silencing; Tubulin (or Actin) was used as loading control.

As previously mentioned, Spindly depleted cells are defective in division and proliferation processes, due to the role of Spindly in mitosis. Therefore, to exclude the possibility that the phenotype defined above was related to defects in cell proliferation, we synchronised cells in S phase by administrating Hydroxyurea (HU) (1mM) for 24 hours and repeated the scratch assay. The treatment allowed us to study cell migration in cells that are neither dividing nor proliferating.

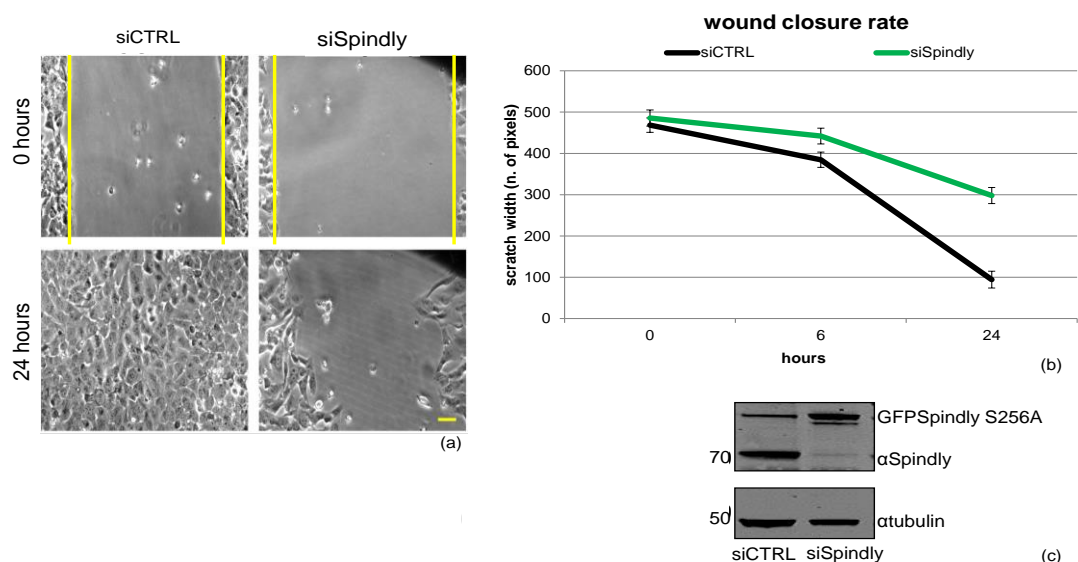
In those cells the lack of Spindly would generate a phenotype strictly related with its non-mitotic function. Control or HU treated cells were imaged for 24 hours (Fig. 5.5, (a)) and then kymographic analysis of the movies were carried out to extract velocities values from the linear movement of the cells (Fig. 5.5, (c)). The synchronisation and the silencing of Spindly were confirmed by Western blot analysis (Fig. 5.5, (b)).



**Figure 5. S phase synchronisation confirms lower migration speeds in Spindly depleted cells.**

(a) Still images of time-lapse analysis comparing control cells (either siCTRL or siSpindly; left hand-side) and hydroxyurea (HU, 1mM) treated cells (either siCTRL or siSpindly; right hand-side). 20X magnification. Scale bar 50  $\mu$ m. (b) Western blot of U2OS control or Spindly depleted cells upon either control or HU treatment (1mM) to confirm S phase synchronisation. Geminin was used as S phase marker. Tubulin was used as loading control. (c) Kymograph analysis of the time-lapse experiments obtained using the Volocity software. Scale bar = space (s): 33  $\mu$ m; time (t): 2 hours.

This experiment provides further evidence that Spindly depleted cells are intrinsically defective in cell migration, and that the defects we measure are not produced by errors in cell division created by Spindly depletion. Interestingly, we did not register differences in the slope obtained from the kymograph analysis of control and HU treated cells (Fig. 5.5, (c)), suggesting that cell divisions and/or density do not contribute greatly to the speed of cell migration. We therefore can confirm that Spindly plays a role in the cell migration process, a totally new function never described to date. This non-mitotic function could be due to its association with the motor complex dynein/dynactin to help the motor exerts its function in cell movement; however, our data cannot necessarily connect Spindly to dynein/dynactin in this context. Nevertheless, is important to notice that, when we tried to rescue the migration defects expressing the GFP-Spindly mutant construct, which impairs the binding of Spindly to dynactin (S256A; see **Chapter 3**), we were not able to rescue the cell movement rate to control levels. Cells expressing only the exogenous copy of the mutant protein were still slower compared to control cells (Fig. 5.6).



**Figure 5. 6. Expression of GFP-Spindly S256A does not rescues migration defects.**

(a) Still images from time-lapse analysis showing cell migration experiment in U2OS GFP-Spindly S256A Spindly-depleted cells compared to U2OS GFP-Spindly S256A siRNA control cells. Scale bar 50  $\mu$ m. (b) Quantification of the width of the scratch in U2OS GFP-Spindly S256A control and depleted cells over time. Data indicate the mean  $\pm$  standard deviation (represented by error bars) from at least 3 independent experiments. (c) Western blot of same

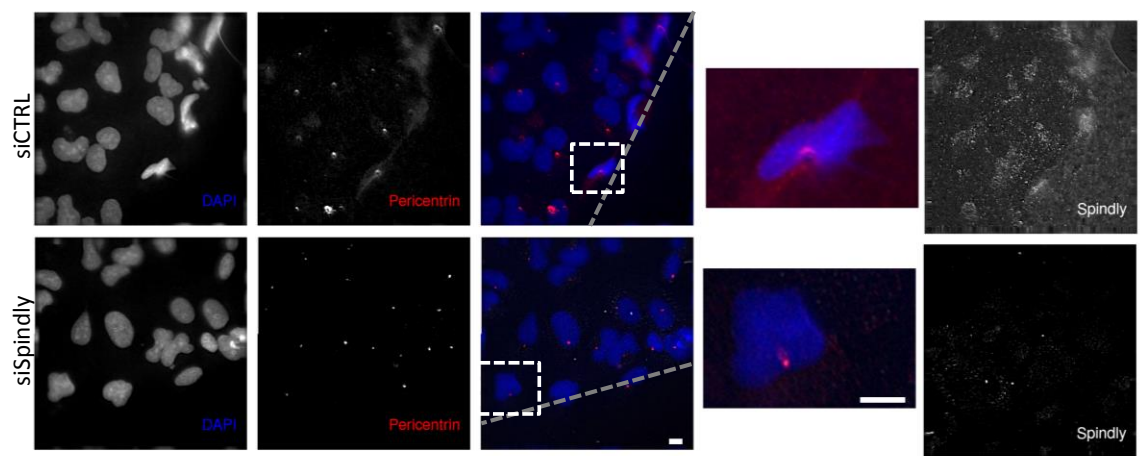
populations of cells confirms the silencing and the GFP expression; Tubulin was used as loading control.

According to this data we can confirm that Spindly is playing a role in cell migration that could be associated with the dynein/dynactin motor complex; this interplay is likely to occur through a mechanism similar to that one previously shown for the SAC stripping process in mitosis.



## 5.4 Spindly depletion does not affect MTOC reorientation

A well-studied step in the wound repair process is the reorientation of centrosomes (or MTOC) to a position between the leading edge and the nucleus (Gundersen and Bulinski, 1988), a crucial activity that sets the direction of migration. Defects in dynein or dynactin expression block the MTOC reorientation process completely (Palazzo *et al.*, 2001). Given that our previous studies have revealed high similarity in cell migration defects between Spindly depleted cells and dynactin depleted cells, we wanted to verify whether Spindly was also required for reorientation of the centrosome. Reorientation normally occurs within the first two hours upon wounding (Palazzo *et al.*, 2001), so we scratched a monolayer of U2OS cells, fixed and stained with Pericentrin to visualise centrosomes (Fig. 5.7).



**Figure 5. 7. Spindly depletion does not affect centrosome positioning.**

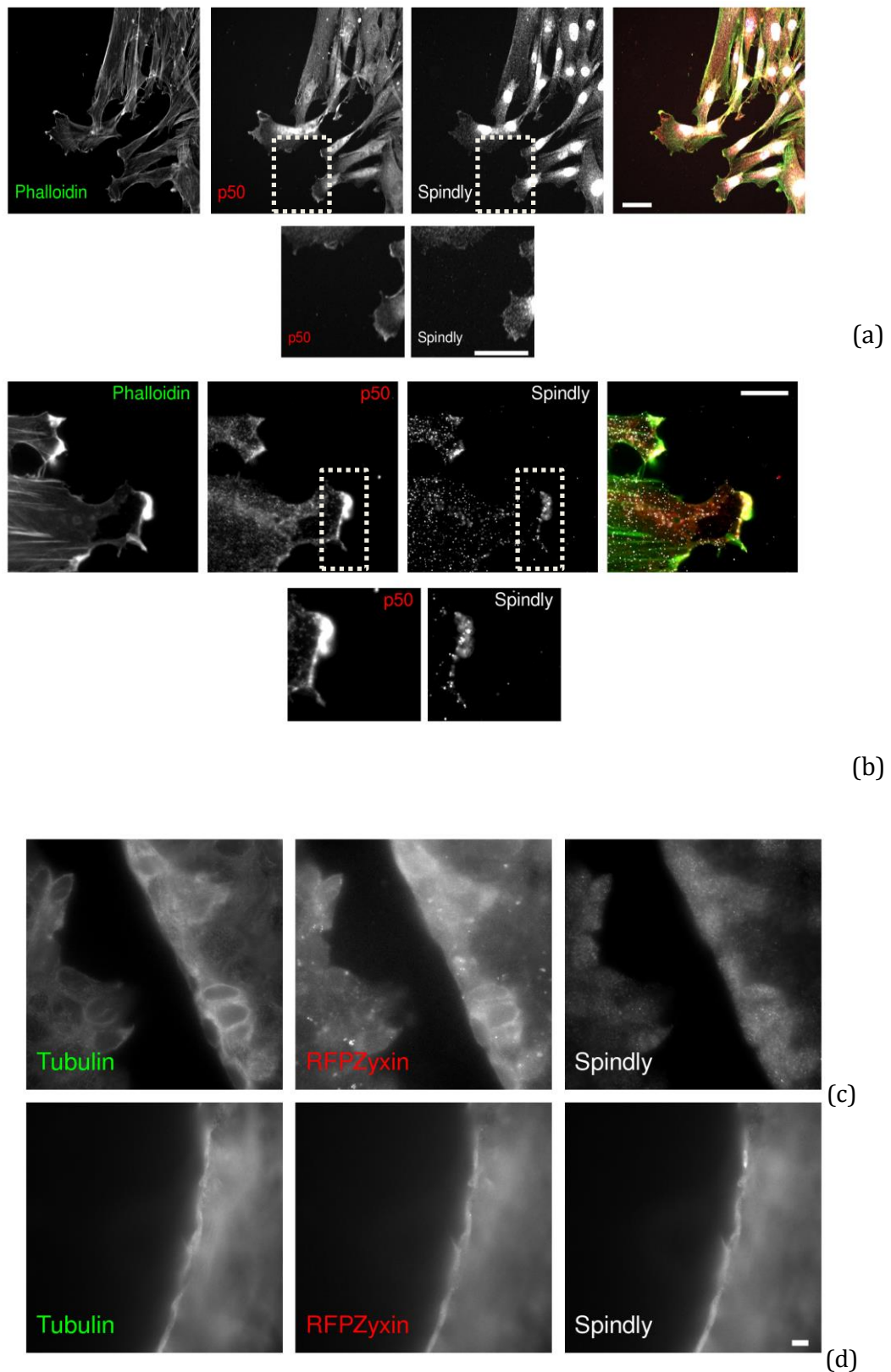
Immunostaining of U2OS cells fixed four hours upon wounding (edge indicated by dotted grey line): nuclei (DAPI, blue), centrosomes (anti-Pericentrin, red). Grey dotted line indicates the cell front. Details represent magnified images derived from the boxed areas (dashed white box) for two channels (DAPI and Pericentrin). 40X magnification. Scale bar 10 $\mu$ m. Right hand side of the panel is anti-Spindly staining to show the effectiveness of the silencing (anti-Spindly, white).



This assay did not reveal any striking defective phenotype or differences in centrosome positioning upon Spindly depletion (measurements were performed to confirm the absence of defects in cells lacking Spindly ( $n \geq 35$ ), graphs not shown). This suggests that Spindly is not involved in the polarisation of the cell to set the axis to direct the migration process. It could instead be recruited at the leading edge in a secondary step to facilitate other processes (as discussed further in this chapter).

## **5.5 Localisation of Spindly during cell migration**

To study the behaviour of cytoplasmic Spindly during cell migration in more detail, we immunostained migrating cells: human fibroblasts, HaCaT cells, or mouse embryonic fibroblasts (MEFs) (Fig. 5.8; (a)/ (b), (c) and (d) respectively).



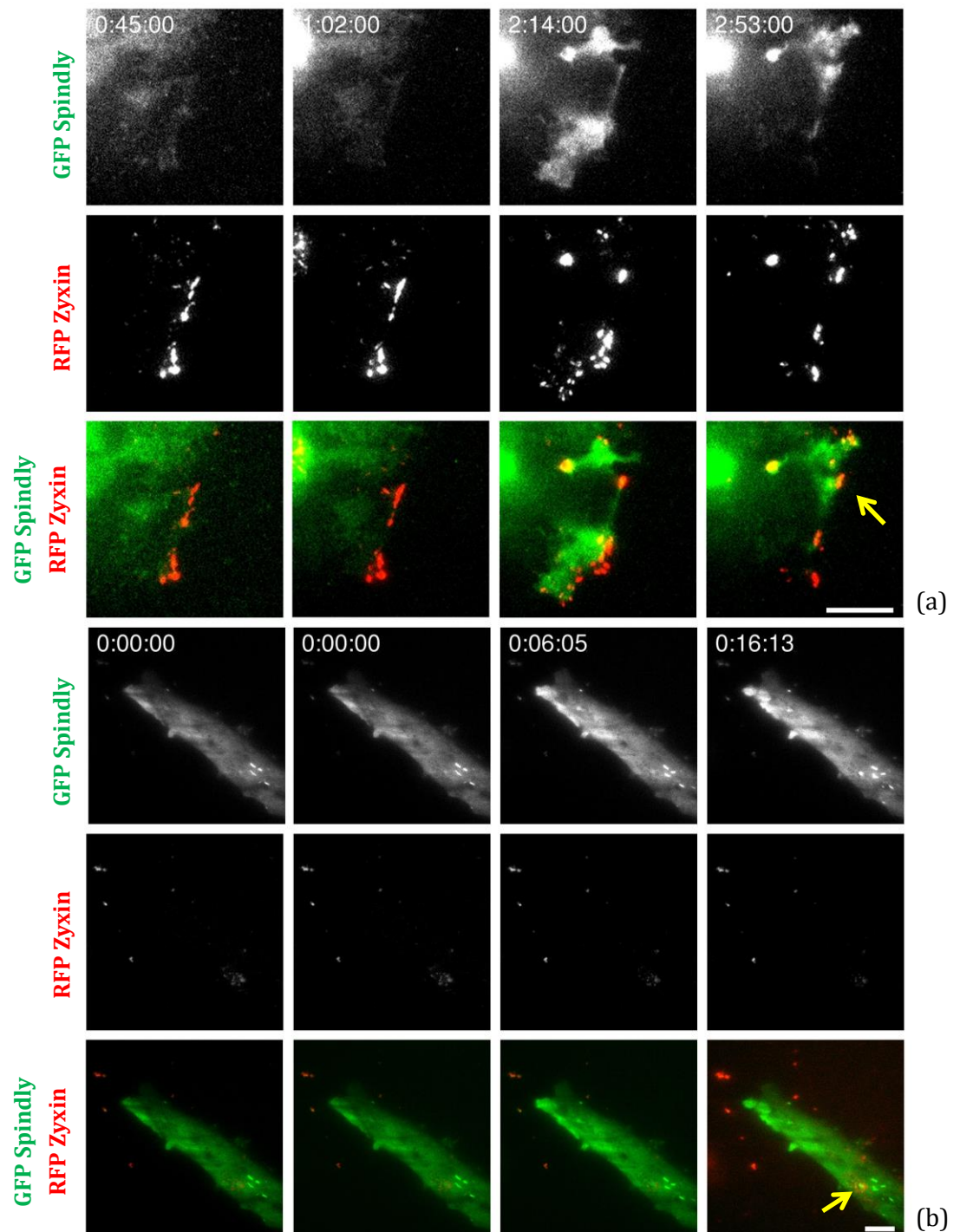
**Figure 5. 8. Spindly is recruited to the leading edge and to focal adhesions upon scratching.**

(a) Immunofluorescence of Human fibroblasts wounded for at least eight hours before fixation showing: actin (Phalloidin, green), dynactin (anti-p50, red) and Spindly (anti-Spindly, white). 20X magnification. Scale bar 10  $\mu$ m. (b) 60X magnification of cells as (a). Scale bar 10  $\mu$ m. Details underneath each panel ((a) and (b)) represent magnified images derived from the boxed areas (dashed white box). HaCAT cells (c) or primary Mouse embryonic fibroblasts (d) were

scratched and fixed after eight hours. 20X magnification. Staining shows: Tubulin (anti-Tubulin, green), RFP-Zyxin (red), Spindly (anti-Spindly, white). Scale bar 10  $\mu$ m.

We detected a clear enrichment of Spindly at the leading edges (within lamellipodial protrusions, as highlighted from the boxed areas in figure 5.8 (a) and (b)) of moving human fibroblast migrating towards the other edge of the wound. Dynactin (p50 subunit) co-localised with Spindly at these sites, an enrichment of the dynein-complex consistent with previous results showing a direct role for this motor in cell movement (Dujardin *et al.*, 2003). Furthermore, Spindly was enriched also at the leading edge MEFs (Fig. 5.8 (d)), co-localising with RFP-Zyxin, a specific marker of focal adhesions (Reinhard *et al.*, 1999). For HaCAT cells instead we could not registered a clear enrichment at the leading edge but only an upregulation in the leading edge (Fig. 5.8 (c)).

To further analyse leading edge localisation of Spindly and its dynamics, we co-transfected both U2OS cells and human fibroblasts with GFP-Spindly and RFP-Zyxin constructs. Upon scratch wounding, we followed the cells by TIRF microscopy (Fig. 5.9). Imaging was performed by Dr. Eric Griffis (University of Dundee).



**Figure 5. 9. Localisation of Spindly at the leading edge during wound healing.**

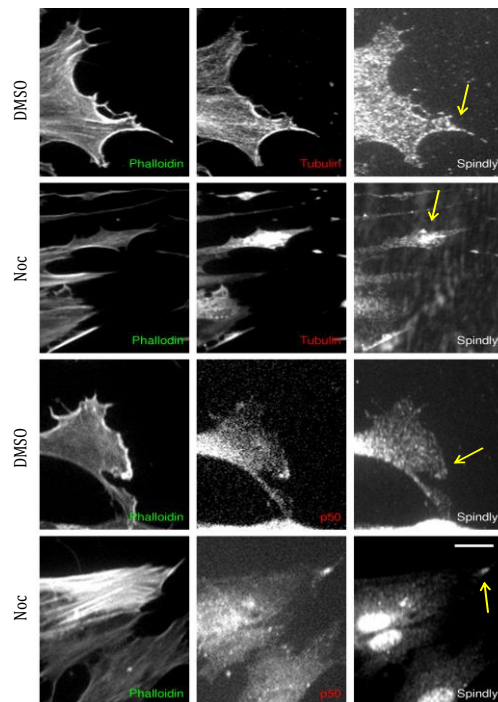
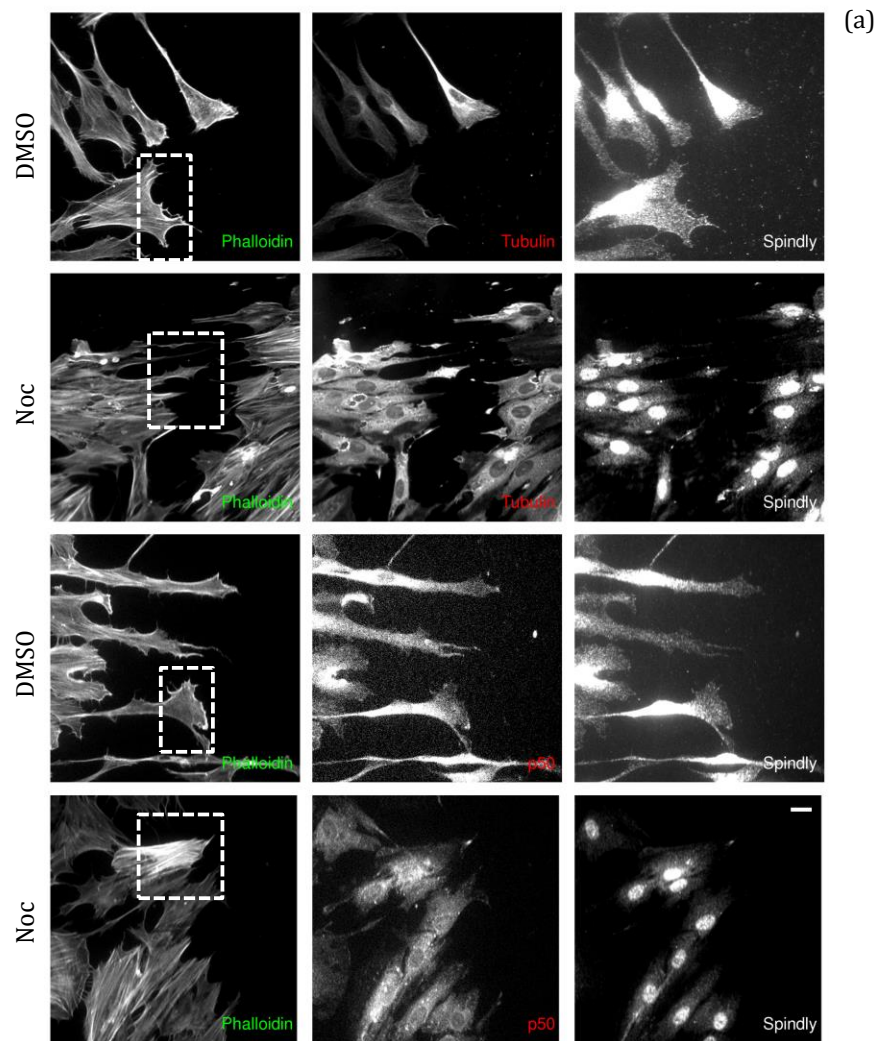
(a) U2OS cells or (b) human fibroblasts were transiently co-transfected with GFP-Spindly (green) and RFP-Zyxin (red). Cells were followed over time under a TIRF microscope. Yellow arrows indicate co-localisation of Spindly with Zyxin. Scale bar 10  $\mu$ m. Imaging performed by Dr. E. Griffis (University of Dundee).

Imaging in figure 5.9 confirmed the recruitment of Spindly to the leading edge in actively moving cells where it co-localises with the focal adhesion marker Zyxin. We have noticed that Spindly co-localised with Zyxin only significantly after migration and Zyxin redistribution had begun; this indicates that Spindly could be involved in the later stages of focal adhesion maturation or turnover (Nagano *et al.*, 2012).

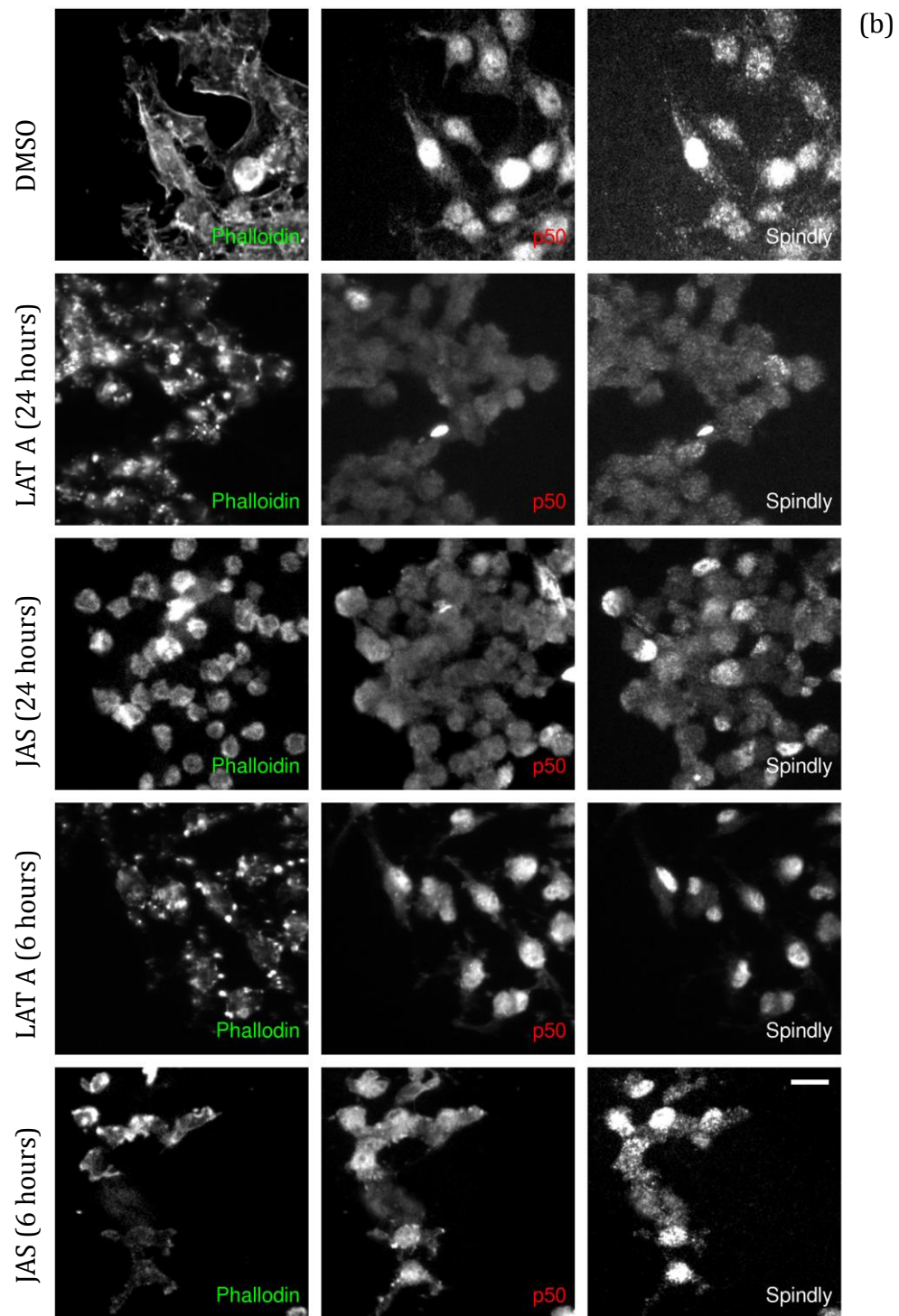
## **5.6 Spindly recruitment at the cell front is based on the presence of actin filaments**

The actin cytoskeleton is directly and dynamically involved in cell migration (Le Clainche and Carlier, 2008). Polarization of actin filaments and their re-organisation at the leading edge are two crucial steps promoting cell movement and thus are highly organised (Le Clainche and Carlier, 2008). Several proteins assemble at the leading edge in order to promote membrane protrusion, adhesion formation and contact with the extracellular-matrix (Sastry and Burridge, 2000). As well as actin filaments, microtubules are also involved in the internal organisation of the cell, playing a direct role in cell shaping and mechanics. Their capacity to regulate cell polarity relies on the presence of microtubule-associated proteins that control microtubule nucleation and dynamics (Etienne-Manneville, 2013). Given the high importance of both systems in cell migration and polarisation, a common belief is that there is a functional crosstalk between microtubules and actin filaments (Rodriguez *et al.*, 2003).

To investigate the specificity of Spindly recruitment to the leading edge and how it gets localised there, we perturbed either microtubules, by administrating Nocodazole (as MT depolymerising agent), or actin filaments, by adding either Latrunculin A (LAT A) or Jasplakinolide (JAS), and looked at Spindly localisation (Fig. 5.10). Latrunculin A blocks actin polymerisation causing depolymerisation (Coue *et al.*, 1987) while treatment with Jasplakinolide *in vivo* disrupts actin filaments inducing polymerisation of monomeric actin into amorphous masses (Bubb *et al.*, 2000)







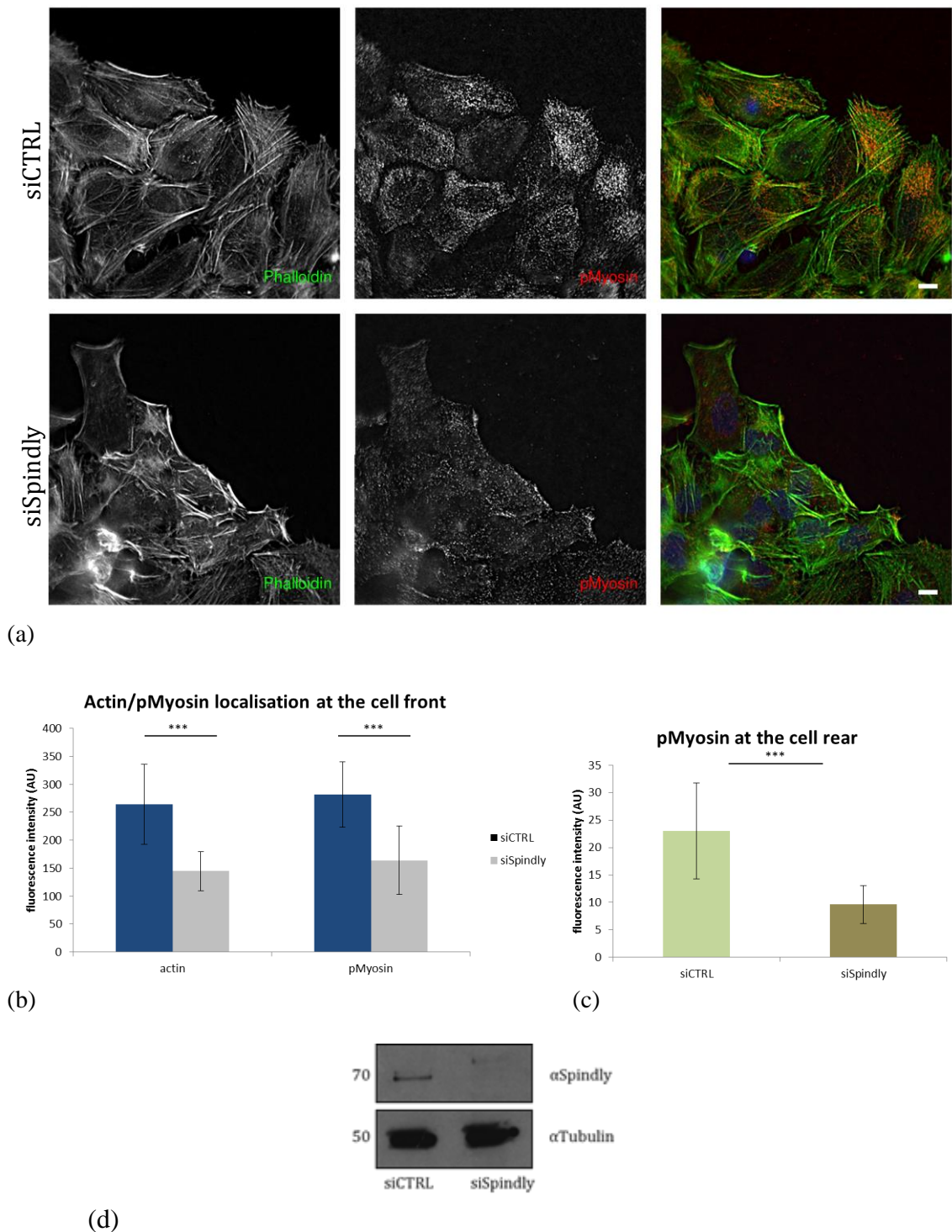
**Figure 5. 10. Spindly and dynactin localisation at the leading edge is not dependent from microtubules.**

Immunofluorescence of human fibroblasts treated with (a) Nocodazole (Noc, 100 nM) for 10 hours, or with (b) Latrunculin (LAT A) or Jasplakinolide (JAS) (100nM) for either 6 or 24 hours prior to fixation. DMSO was used as negative control (both panels (a) and (b)). **Panel (a):** actin filaments (Phalloidin, green); microtubules (anti-Tubulin, red) or dynactin (anti-p50, red); Spindly (anti-Spindly, white). The bottom side of **panel (a)** shows magnified images derived from the boxed areas (dashed white box). Yellow arrows indicate accumulation of actin, p50 and Spindly at focal adhesion sites **Panel (b):** actin filaments (Phalloidin, green); dynactin (anti-p50, red); Spindly (anti-Spindly, white). 20X magnification. Scale bar 20  $\mu$ m.

Overnight treatment of U2OS cells with Nocodazole did not affect Spindly recruitment to actin-rich sites at the cell periphery (Fig. 5.10, (a), yellow arrows). On the other hand, when we affected the actin filament network by administrating either LAT A or JAS, we registered defects in localisation (Fig. 5.10, (b)). In this scenario however it is important to point out that these actin inhibitors dramatically affect the cell morphology so the defects registered could be related to side effects due to the treatment. Therefore there is the possibility that the defects registered in our experiments could be not specific. Thus, it would be worth to repeat this study using a new cocktail of actin drugs recently developed that allow to arrest actin dynamics without affecting the cell structure (by pre-incubating cells with Y27632 (ROCK inhibitor) and then adding LAT A and JAS for short period of time (Peng *et al.*, 2011)). On the other side, to analyse deeper the interplay between actin and Spindly in cell migration we could also study actin dynamics upon Spindly depletion. For instance it would be interesting to overexpress Rho GTPase and look at stress fibres formation and dynamics upon siRNA of Spindly.

## **5.7 Spindly depletion affects actin and phospho-Myosin distribution in migrating cells**

The cell migration cycle includes protrusion, adhesion, contraction and retraction, and all of the steps are tightly coordinated. After protrusion, translocation of the cell body occurs thanks to a coordinated contraction of the actomyosin cytoskeleton, more specifically thanks to phosphorylation of Myosin 2 mediated by Myosin regulatory light chain kinases (MLCKs), a kinase that allow for separation of the diverse Myosin 2 functions by phosphorylating distinct Myosin contractile modules (Lauffenburger and Horwitz, 1996). At the lamellipodium, MLC kinases together with Rho kinase (ROCK) have been demonstrated to regulate the extension of the region and the formation of focal complexes by phosphorylating Myosin 2 inducing in this way its activation and subsequent regulation of stress fibres and focal adhesions formation (Totsukawa *et al.*, 2004). Given the defects observed in cell migration upon Spindly depletion, we decided to analyse localisation of actin and phospho-Myosin (pMyosin) in U2OS cells depleted of Spindly at the edge of a wound (Fig. 5.11).



**Figure 5. 11. Spindly depleted cells present lower amount of actin and pMyosin.**

U2OS cells were treated with either siRNA for Spindly or control (CTRL) for 96 hours. Wound was performed and after 3 hours cells were fixed and stained. (a) Staining shows: actin filaments (Phalloidin, green); phospho-Myosin (pMyosin, red); Spindly (anti-Spindly, white). Scale bar 10  $\mu$ m. (b) Quantification of the fluorescence intensity of either actin or pMyosin at the cell front in control or Spindly depleted cells. (c) Quantification of the fluorescence intensity of pMyosin at the cell rear in control or Spindly depleted cells. Measurements were performed with the FiJi software ( $n \geq 50$ ) and statistical analysis was performed with Excel. Data indicate the mean  $\pm$  standard deviation (represented by error bars) from at least three experiments and were analysed by Student's *t*-test with significance defined by \*: \*\*\*=  $p < 0.001$ . (d) A sample of the same cell population was harvested and analysed by Western Blotting to confirm the silencing.

Figure 5.11 reveals that upon Spindly depletion both actin polymer and phospho-Myosin levels are reduced. We registered a significant reduction of about 40% in the amount of both markers at the cell front (5.11, (b)) and of about 58% in the level of phospho-Myosin at the cell rear (Fig. 5.11, (c)). This data corroborate our hypothesis that Spindly plays a critical role in cell migration, and it suggests that there is a function for Spindly in the formation of actin polymers and regulation of Myosin activity.

## 5.8 New potential interactors for human Spindly in interphase

As stated earlier, human Spindly has never been studied in non-mitotic cells, so there are no clues on how its recruitment to cytoplasmic foci could be facilitated and what binding partners it might have at the leading edge. Consequently, it was decided to investigate potential interactors, taking advantage of mass spectrometry technique.

We exploited the untreated HEK293 cells already processed for the former described MS study (reported in Chapter 4- Paragraph 4.5 / Fig. 4.8). We screened the dataset for actin-related proteins that could explain the presence of Spindly at the cell front and therefore could give us new potential interactors. Specifically we took into account the number of peptides identified for each protein and the intensity value at which they were identified. We compared the list obtained for the “Spindly IP” dataset with the “IgG” dataset (our negative control) and proceeded with the analysis only for those proteins that gave an intensity value equal to zero in the “IgG” dataset.

A summary of candidates that could be implicated in the recruitment and/or in the functioning of Spindly in the cell movement process is presented in figure 5.12. Functions of the proteins reported in the table (Fig. 5.12 (a)) were confirmed from the UniProt Human Database (Fig. 5.12 (b)) and a network analysis was performed using the String-bd software (Fig. 5.12 (c)) to link those proteins according to the information already available on physical and functional protein-protein interactions, proved either by experimental data or computationally prediction (Jensen *et al.*, 2009).

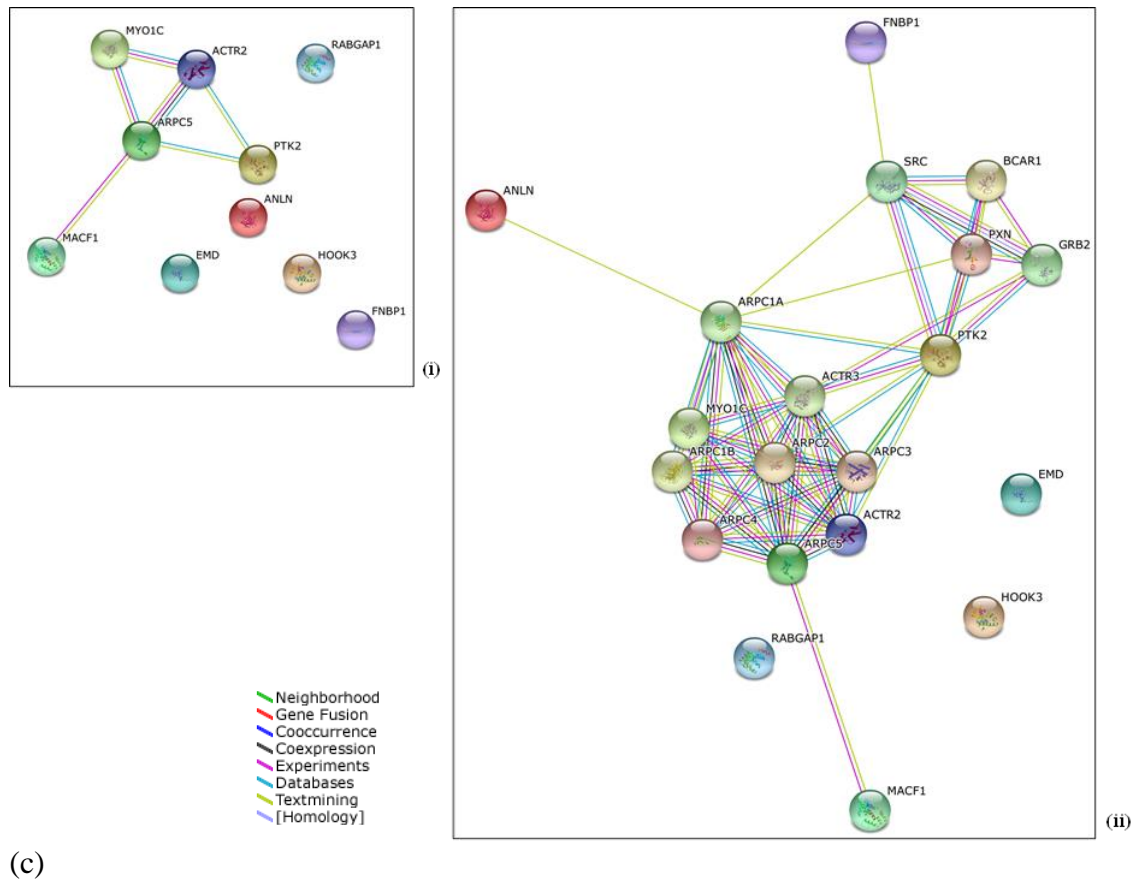
<b>ACTIN ASSOCIATED PROTEINS</b>	<b>PEPTIDES</b>	<b>INTENSITY Spindly-IP</b>	<b>INTENSITY Rb IgG</b>
MACF1	32	1045200	0
Myosin IC	21	1787900	0
ANILLIN like protein	11	3305500	0
Arp 2/3	6	6866880	0
FAK	3	4026500	0
Rab GAP 1	3	2278500	0
HOOK3	3	1070200	0
Formin BP4	2	1368800	0
Arp 2/3 subunit 5	2	561970	0
EMERIN	2	555000	0

(a)

<b>Protein name</b>	<b>Protein function</b>
<b>ANLN</b>	anillin, actin binding protein; Required for cytokinesis. Essential for the structural integrity of the cleavage furrow and for completion of cleavage furrow ingression.
<b>HOOK3</b>	hook homolog 3 (Drosophila); May regulate clearance of endocytosed receptors such as MSR1. Participates in defining the architecture and localization of the Golgi complex.
<b>MYO1C</b>	myosin IC; Myosins are actin-based motor molecules with ATPase activity. Unconventional myosins serve in intracellular movements. Their highly divergent tails are presumed to bind to membranous compartments, which would be moved relative to actin filaments.
<b>MACF1</b>	microtubule-actin crosslinking factor 1.
<b>EMD</b>	emerin. Stabilizes and promotes the formation of a nuclear actin cortical network. Stimulates actin polymerization in vitro by binding and stabilizing the pointed end of growing filaments. Inhibits beta-catenin activity by preventing its accumulation in the nucleus. Links centrosomes to the nuclear envelope via a microtubule association.
<b>RABGAP1</b>	RAB GTPase activating protein 1. May act as a GTPase-activating protein of RAB6A. May play a role in microtubule nucleation by centrosome. May participate in a RAB6A-mediated pathway involved in the metaphase-anaphase transition.
<b>ACTR2</b>	ARP2 actin-related protein 2 homolog. Functions as ATP-binding component of the Arp2/3 complex which is involved in regulation of actin polymerization and together with an activating nucleation-promoting factor (NPF) mediates the formation of branched actin networks.
<b>ARPC5</b>	actin related protein 2/3 complex, subunit 5. Functions as component of the Arp2/3 complex which is involved in regulation of actin polymerization and together with an activating nucleation-promoting factor (NPF) mediates the formation of branched actin networks.
<b>FAK</b>	PTK2 protein tyrosine kinase 2. Non-receptor protein-tyrosine kinase that plays an essential role in regulating cell migration, adhesion, spreading, reorganization of the actin cytoskeleton, formation and disassembly of focal adhesions and cell protrusions, cell cycle progression, cell proliferation and apoptosis.
<b>FNBP1</b>	Formin-binding protein. It may act as a link between RND2 signaling and regulation of actin cytoskeleton. Required to coordinate membrane tubulation with reorganisation of the actin cytoskeleton during the late stage of clathrin-mediated endocytosis. Also it enhances actin polymerisation via the recruitment of WASL/N-WASP, which in turns activates the Arp2/3 complex.

(b)





**Figure 5. 12. Identification of novel Spindly binding partners in interphase cells.**

(a) Table reporting molecular candidates enriched in samples immunoprecipitated with anti-Spindly antibody Rabbit IgG was used as a negative control. (b) Summary of the functions of the enriched proteins (adapted from information obtained from UniProt Database, [www.uniprot.com](http://www.uniprot.com)). (c) STRING network Diagram representing the network for either only the Spindly-IP enriched proteins (i) or for the Spindly-IP enriched proteins plus their previously reported binding partners (ii). This “evidence view” was obtained from String-bd.org, a database of known and predicted protein interactions derived from different sources. Colour legend is reported in the left hand-side of the panel to explain the different connection lines. Single lines mean confidence of interaction, whereas double lines indicate evidence for interaction. Each protein is represented by a node.

The sub-set of proteins obtained from the mass spectrometry analysis includes several known regulators of the actin and microtubule cytoskeleton (as reported in the table in figure 5.11, (b)). Additionally, in the list of enriched proteins for the Spindly-IP dataset, we also found other interesting proteins involved in cell migration but we did not consider them in our analysis since their IgG value was not equal to zero.



The presence of proteins already described involved in directed cell movement, cell polarisation or adhesion formation/turnover, reinforces the hypothesis that Spindly can play a role in this process in association with actin and/or microtubules, as was already suggested from results obtained earlier in this chapter.

## 5.9 Discussion

The cytoskeleton is made up of three different networks: microtubules (MTs), actin filaments and intermediate filaments (IFs). These three structures cooperate in many different cellular processes, allowing a cell to respond properly to a wide range of stimuli (Wickstead and Gull, 2011). Among these processes, cell migration directly involves the whole network to ensure first a change in the cell morphology, in response to specific signals, and then actin assembly at the leading edge, to promote extension of the membrane. Finally, the disassembly of adhesions and actomyosin contraction at the trailing edge allows for the forward movement of the cell body (Le Clainche and Carlier, 2008). Actin polymerisation dynamics are closely regulated by many different signalling molecules and a legion of binding proteins (Pollard and Borisy, 2003). Dynamic microtubules have also been shown to associate with focal adhesions in migrating cells as well as to be directly involved in cell movement (Vasiliev *et al.*, 1970) (Kaverina *et al.*, 1998). They perform an important role in controlling focal adhesion turnover and localise to the areas near these adhesion sites as direct players in the microtubule organisation or stabilisation at the leading edge (Stehbens and Wittmann, 2012). Even though actin has a main role in the organisation of the leading edge of the cell, microtubules are likewise required to maintain polarisation; the actomyosin network pushes microtubules rearwards in the lamella of motile cells where microtubule tips can be loaded with factors that regulate actomyosin activity (Salmon *et al.*, 2002) (Rogers *et al.*, 2004).

In this chapter we defined a striking role for human Spindly in cell migration; we proved an involvement of the protein in directed cell movement, describing a phenotype highly similar with those already delineated for dynein, dynactin and Lis1 (Faulkner *et al.*, 2000) (Dujardin *et al.*, 2003). Spindly depleted cells showed a reduction in the rate

of cell migration upon wounding and staining of migrating cells revealed the protein specifically recruited at the leading edge in an actin-dependent manner.

Also, we registered a reduction in both actin polymer and phospho-Myosin levels upon depletion of Spindly that suggests a role for Spindly in the formation of actin polymers and in the regulation of Myosin activity in cell migration.

As already pointed out in **Chapter 3**, Spindly is a novel adaptor for the minus-end motor complex dynein/dynactin; here we showed that this could be true not only in mitotic cells but also in interphase cells. Our results led us to propose a model where Spindly is involved in the enhancement of the dynein/dynactin targeting or motor processivity in order to promote cargo loading and/or tension generation at focal adhesion sites. The fact that we did not see centrosome positioning defects in our studies (as previously reported for dynein depleted cells (Palazzo *et al.*, 2001) (Levy and Holzbaur, 2008)) and that Spindly was enriched at focal adhesions late in their formation could suggest that the association of Spindly with the leading edge happens downstream of MTOC reorganisation and that is involved in regulation of focal adhesion maturation and turnover. According to this view, we see Spindly as a potential MT/actin filament cross-linker, crucial for proper cytoskeleton interplay during cell migration. The importance of MT/actin filament crosstalk is related to the capacity to deliver molecules or membrane components to the leading edge (Watanabe *et al.*, 2005); this mechanism has been described based on cell polarisation, more precisely on MTOC reorganisation that requires Cdc42 (a small Rho GTPase) and dynein (Palazzo *et al.*, 2001). Dynein and dynactin are recruited to the cell front from where they could generate pulling forces to move the centrosome toward the leading edge, this would allow placing the Golgi apparatus and the endocytic recycling compartment in front of the nucleus to polarise membrane trafficking toward the leading edge. Moreover, dynein

has been demonstrated to be involved in Golgi dynamics and structure maintaining in association with dynactin (that associates with spectrin (Holleran *et al.*, 2001)) suggesting a role for it in the trafficking of cargos at the front of the cell (Etienne-Manneville, 2013). Therefore Spindly could either help the recruitment of dynein at the leading edge or promote the process of cargo loading and enhancing the processivity of the motor.

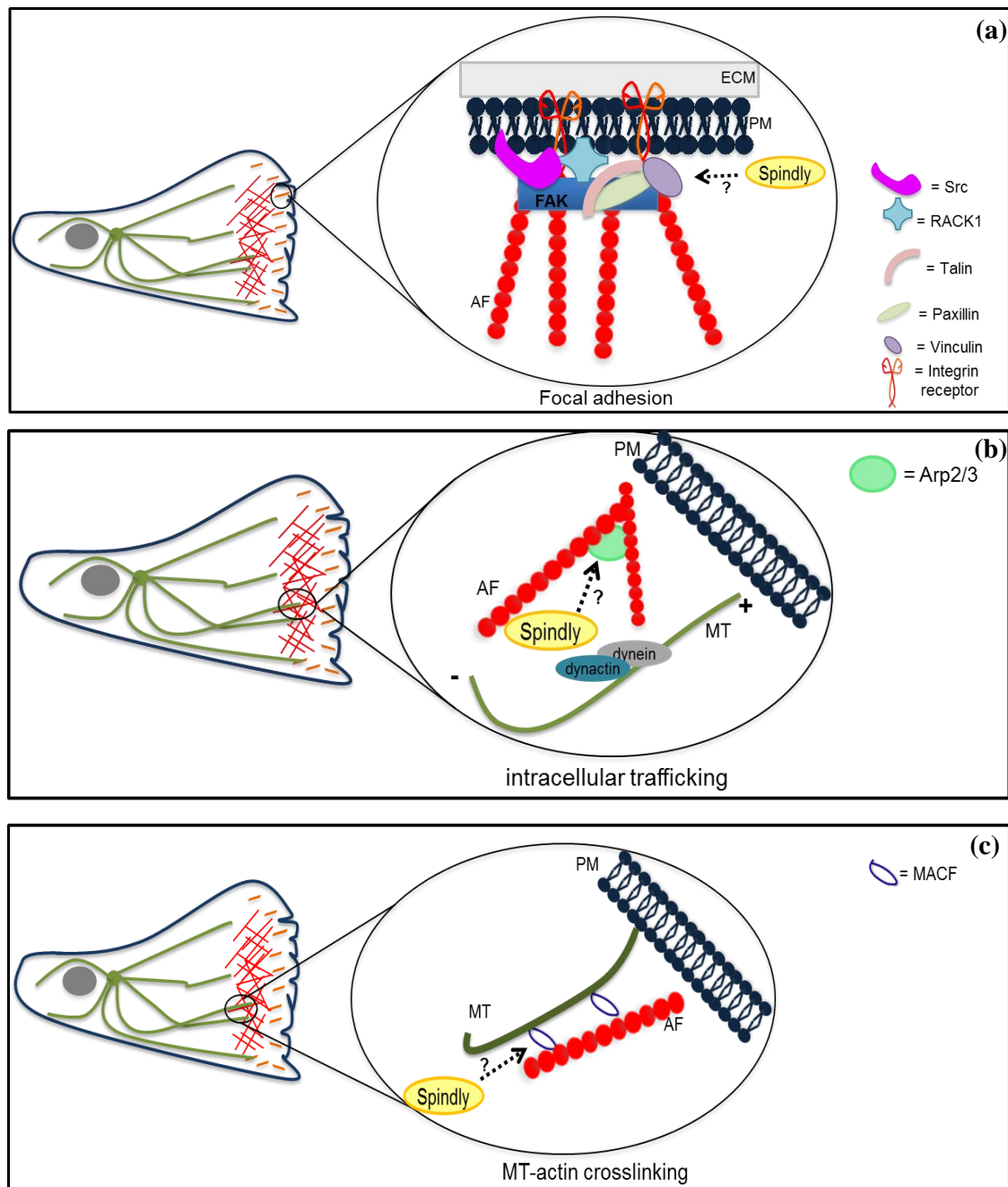
From our mass spectrometry analysis we identified a list of potential binding partners of high interest. Several of them are cytoskeletal regulatory proteins also involved in focal adhesion formation, assembly and dynamics; they contribute to the reorganisation of the cytoskeleton network upon wounding corroborating our hypothesis that Spindly can exert a role in cell motility. Interesting was, for instance, the presence of MACF1 (microtubule actin cross-linking factor 1, also called ACF7), a protein that plays a role in stabilisation of microtubules at the leading edge and also controls focal adhesion assembly and dynamics (Kodama *et al.*, 2003). In the same way, Anillin attracted our attention since it has been described in the regulation of actin cytoskeleton dynamics as well as in the cross-talk between actomyosin cytoskeleton and microtubules (Tian *et al.*, 2015). Spindly could also immunoprecipitate other proteins directly involved in the focal adhesion assembly process. Focal adhesion kinase (FAK) is crucial for the turnover of cell contacts. It is typically located at focal adhesions and phosphorylated upon integrin engagement (Burridge *et al.*, 1992) (Arold *et al.*, 2002); it mediates the link between the extracellular matrix (ECM) and the cytoplasmic cytoskeleton as does Emerin, (in our Spindly-IP as well) by interacting with the nuclear lamins (Clements *et al.*, 2000) (Chang *et al.*, 2013). Curiously, we identified two subunits of the Arp2/3 complex, a major player in the regulation of actin cytoskeleton since it stimulates polymerisation of new actin filaments (Pollard, 2007). The Arp2/3 complex has been found at the leading edge of motile cells (precisely within

lamellipodia, where we also observe Spindly) and it is involved in the establishment of cell polarity (Magdalena *et al.*, 2003). Finally, it was intriguing to find RABGAP1, the GTPase activating protein of Rab6, a marker for vesicle trafficking that promotes microtubule-mediated transport to the cell periphery together with kinesin-1 or dynein via BicD (Grigoriev *et al.*, 2007) (Hoogenraad *et al.*, 2003).

It remains to be determined what exactly its function is; Spindly could be operating through its regulation of dynein/dynactin, it could have a direct role in regulating actin polymerisation or focal adhesion function, or it could play a combination of these activities.

Although experiments conducted in this study suggest a new role for Spindly in cell migration in interphase cells in relation with the motor complex dynein/dynactin, further analyses are still needed to fully understand the mechanism behind its recruitment at the leading edge and its mechanism of action. Our data support different potential functions of Spindly in cell migration. On one side, cortical Spindly could be directly modifying actomyosin activities to promote protrusion or focal adhesion maturation and/or turnover (as hinted from its specific localisation and recruitment at sites, potentially by an association with FAK) (Fig. 5.13 (a)). On the other side, Spindly could be playing a function in association with the dynein/dynactin motor complex at the leading edge, potentially mediating the intracellular trafficking of actin filaments from the front to the rear to support actin turnover (as suggested from the interaction with Arp2/3, Anillin and Formin Binding Protein) (Fig. 5.13 (b)). Finally, the localisation of Spindly at the leading edge and at the MT-plus ends suggests a potential role in the stabilisation of MTs at the leading edge and/or in the physical linking of the two cytoskeleton networks, MTs and actin filaments (as suggested also from the interactors MACF1) (Fig. 5.13 (c)).

These hypotheses are not mutually exclusive and they would need to be tested to understand the dynamics of these interactions.



**Figure 5. 13. Schematic model for potential functioning of Spindly in cell migration.**

Spindly could be: (a) involved in focal adhesion maturation and/or turnover; (b) interacting directly with actin filaments and maybe mediating their trafficking in association with dynein/dynactin; (c) mediating the crosslinking between actin filaments and microtubules, potentially in relation with MACF1. PM: plasma membrane; MT: microtubules; AF: actin filaments.

## **6 . Discussions and future perspective**

## 6.1 Spindly in mitosis

Spindly was discovered due to its role in mitotic progression. Cells depleted of this protein were incapable of proceeding through cell division but were arrested in metaphase and were eventually undergoing either apoptosis or mitotic slippage with progression into anaphase and poor outcomes for the daughter cells. The studies that followed the initial discovery of Spindly shed light on other important details about its function in mitosis, pointing out why the lack of Spindly induced such a strong mitotic phenotype, in relation with its role in the regulation of chromosome alignment and spindle assembly checkpoint silencing and also identifying a critical region involved in these functions (see **Chapter 1- Paragraph 1.3**) (Griffis *et al.*, 2007) (Gassmann *et al.*, 2008) (Chan *et al.*, 2009) (Gassmann *et al.*, 2010) (Barisic *et al.*, 2010). Therefore the emergent model places Spindly as central in the progression of mitosis and in the coordination of KT-MT binding with SAC silencing process.

The aim of this thesis was to investigate in depth the role of Spindly in mitosis. We focused on the interaction with the dynein/dynactin motor complex and on the functioning of Spindly in relation with the mitotic checkpoint, following the idea that Spindly could play a part not only in its silencing but in establishing or maintaining the signalling of the SAC until bi-orientation has been achieved.

### 6.1.1 The interaction between Spindly and the dynein/dynactin motor complex

Kinetochore localisation of dynein is needed for poleward movement of chromosomes in early mitosis upon interaction with microtubules (Yang *et al.*, 2007); then once bi-orientation is achieved dynein leaves the kinetochore and streams along microtubules to the spindle poles stripping away the components of the SAC to silence the signalling and promote anaphase onset (Howell *et al.*, 2001). The presence of



Spindly at kinetochores is fundamental for the correct localisation of the dynein/dynactin motor complex (Griffis *et al.*, 2007) (Gassmann *et al.*, 2008) (Chan *et al.*, 2009) (Gassmann *et al.*, 2010) (Barisic *et al.*, 2010) (Cheerambathur *et al.*, 2013).

In this work, we have shown for the first time an interaction between Spindly and the dynein/dynactin motor complex. Results obtained revealed that Spindly can pull-down both dynein and dynactin (see **Fig. 3.2** / **Fig. 3.8**). Interestingly, it has been demonstrated a specific binding between Spindly and the p150 subunit dependent on the “Spindly box” region (see **Fig. 3.5** / **Fig. 3.8**); thus this subunit is likely to be a link between Spindly and the motor complex.

Recent publications pointed out that dynactin is not sufficient to activate the processivity of dynein, but instead motility requires an adaptor protein that can link both complexes (Schlager *et al.*, 2014). Among the proteins tested, Spindly was described to have the capacity to promote dynein/dynactin processivity (McKenney *et al.*, 2014). Putting together results from these publications and those gathered in this thesis, the hypothesis that Spindly is an important adaptor of the dynein/dynactin motor complex becomes stronger. It is likely that dynein/dynactin and Spindly generate a ternary complex active only in specific sites within the cell and for selective cellular processes. To dissect this association and the assembly process of the complex, additional experiments will follow this thesis. We have begun experiments in collaboration with the Carter lab (LMB, Cambridge), which previously solved the crystal structure of dynein (Carter *et al.*, 2011) and the EM (electron microscopy) structure of the dynein/dynactin complex (Urnavicius *et al.*, 2015), to look at this potential ternary complex. The Carter lab has managed to solve the EM structure of Spindly (although not with a level of resolution where we can identify amino acids), but we have not been able to reconstitute the ternary complex yet (as judged by SEC experiments (data not shown)) using recombinant human Spindly (produced in bacteria or in insect cells), pig

brain dynactin and a recombinant dynein tail complex, suggesting that there has to be an additional layer of regulation. We are currently exploring whether dimerisation/multimerisation of Spindly influences complex assembly (we see binding of dynein/dynactin when Spindly is present at high concentrations on beads, but not in solution). It is also possible that the farnesylation site at the C-terminus drives the protein to locally concentrate on the kinetochore and to promote clustering and subsequently the dynein/dynactin binding, similar to how kinesin Unc104 membrane association promotes its dimerisation and activation (Tomishige *et al.*, 2002). We are therefore making an MBP-Spindly expression construct with a C-terminal leucine zipper from GCN4 to make a dimerised protein that the Carter lab can test.

If we can assemble the Spindly-dynein-dynactin complex, the Carter lab will solve the structure by cryo-EM.

As reported in the Introduction (see **Chapter 1- Paragraph 1.3**), the structure of Spindly is characterised by two coiled-coil regions interspersed with a highly conserved sequence, the ‘Spindly-box’, present in all the species among which Spindly has been identified (Gassmann *et al.*, 2010) (Barisic *et al.*, 2010). The work presented in this thesis showed that single mutation within this region (S256A) specifically impairs the association between Spindly and the p150 subunit of dynactin, but it does not seem to affect the interaction with dynein. This hints that Spindly interacts also with dynein, but independently of dynactin to allow for chromosome alignment to occur properly (Gassmann *et al.*, 2010). Moreover, our collaborators in the Vaughan lab have found that dynein can still stream from kinetochores when the Spindly S256A mutant is expressed, whereas it does not when Spindly is depleted. This result suggests that in the S256A mutant expressing cells dynein reaches the kinetochores and streams away without loading/stripping cargos and it could explain why cells expressing the mutant can still align chromosome properly. These data also show that Spindly interacts with

dynein/dynactin in a manner that is different from BicD, which only interacts with the holo-complex but cannot bind to either complex separately.

Previous data have reported how dynein can play two functions at KT, regulating chromosome alignment and stripping proteins from KT to allow anaphase onset (Howell *et al.*, 2001) (Yang *et al.*, 2007). It has been demonstrated that dynein is present at KT in two states, a phosphorylated one (on the intermediate chain), which interacts with the RZZ complex, and a non-phosphorylated one, which associates with dynactin (Whyte *et al.*, 2008). Therefore, it might be that phospho-dynein binds to KT prior to microtubule attachments and that KT-MT interactions then induce the de-phosphorylation (upon generation of tension) to promote association with dynactin and activation of the stripping process. In this scenario, Spindly could behave as an activator of the motor complex and/or as an anchor for the motor to the RZZ complex. The registered interaction between Spindly and dynein in both immunoprecipitation experiments and in direct *in vitro* binding assays indicate that there is a strong association that is not affected by the S256A mutation. We therefore hypothesise that while phospho-dynein interacts with the RZZ complex through Spindly it is inhibited from streaming but capable of relieving the RZZ-mediated inhibition of forming proper 'end-on' KT-MT attachment. Once dynactin associates with Spindly and the dynein that it is bound, the motor becomes processive and can promote stripping of SAC components.

Further studies will allow us to shed the light on this mechanism. It would be interesting to look at the processivity of the dynein/dynactin motor complex when the Spindly mutant is substituted with the wild-type protein. The Vale lab revealed that this complex is processive on microtubules, but in personal communications with Dr. McKenney, we did find out that Spindly was not as efficient at promoting processivity of dynein/dynactin as BicD. Our IP and Y2H results showed that the S256A mutant

cannot bind to the p150 dynactin subunit, suggesting that this mutant may not be able to support processive motility on microtubules. If we can find conditions that promote the formation of a stable ternary Spindly/dynein/dynactin complex (as previously explained), the Vale lab will retest the WT and S256A Spindly proteins for their ability to promote motor processivity.

### 6.1.2 A role for Spindly in SAC signalling

As previously stated, *Drosophila* Spindly plays a role in SAC silencing; indeed its depletion causes Mad2 accumulation on aligned kinetochores (Griffis *et al.*, 2007). Curiously, in human cells depletion of Spindly did not have the same effect. This indicated that in the absence of Spindly, dynein (which is never brought to the kinetochore under this condition) is not needed to silence the SAC, suggesting that Spindly may reinforce or help to maintain the checkpoint. The goal of this thesis was therefore to investigate a possible active role for Spindly in checkpoint signalling.

Data collected in the present study identified an interaction between Spindly and proteins of the SAC pathway. Spindly was demonstrated to pull down BubR1 and Mps1, two important players of the mitotic checkpoint. BubR1 is part of the MCC complex, the core player of the SAC involved in the direct inactivation of the APC/C (Lara-Gonzalez *et al.*, 2012) (interactions with APC/C components were also observed in our MS analysis). BubR1 also plays a role in the inactivation of the SAC (see **Chapter 1- Paragraph 1.2**), thus the interaction between Spindly and BubR1 could be important either for the activation/signalling of the checkpoint and/or for its inactivation. If the interaction between Spindly and BubR1 is essential, and if we can find a subtle mutation that alters the binding, then we would be able to make a double mutant that will fail to arrest cells in mitosis in spite of its continued presence on kinetochores.

The association with BubR1 was confirmed also by the MS analysis of mitotic cells and by a 'mis-localisation' system that allowed us to verify the binding in cells. Upon mis-localisation to the plasma membrane Spindly was still able to interact with BubR1 as well as with Mps1 and Mad2. The fact that the association with Mps1 and Mad2 was seen only when the SAC proteins were overexpressed suggests that it might be indirect or of low affinity. Further investigations will be carried out in order to verify the potential presence of a complex and the mechanisms behind its assembly, to understand when Spindly starts its association in the SAC pathway. It would be interesting to repeat the size-exclusion chromatography experiment, comparing lysates made from cells treated with Nocodazole or STLC to enrich for cells with different kinetochore statuses (+/- MT attachments) and potentially get different population of interactors.

In the future, the exact nature of the role of Spindly in the SAC signalling pathway needs to be determined; looking at our results we can propose some potential models. Spindly could be a scaffold protein that, after KT-MT attachment, mediates the loading of the SAC components onto a large dynein/dynactin transport complex; so, when Spindly is not removed from kinetochores, it keeps SAC proteins present and the checkpoint active. Alternatively, Spindly could be inhibiting the crucial de-phosphorylation of Knl1 that facilitates SAC silencing. Finding that Spindly co-immunoprecipitates with key phosphatases PP2A and PP1 and the kinase Mps1 suggests that it could have a role in regulating KT phospho-signalling. If this was the case, then the regulation of well-characterised kinetochore phosphosites, which are involved in SAC activation/inactivation, would be altered in cells expressing solely the unstrippable Spindly mutant (S256A).

Finally it could also be that the mere presence of Spindly on the kinetochore is sufficient for maintaining SAC activity. Then we should be able to recapitulate the 'Spindly box' mutant effect by using the FRB-FKBP system, where we use Spindly-

FKBP and a KT protein-FRB. This technique has already been exploited to show that the retention of Mad1 and/or Mps1 is sufficient to arrest cells in metaphase (Jelluma *et al.*, 2010) (Kuijt *et al.*, 2014). We could also use this system to determine which proteins are aberrantly retained on KTs when Spindly is irreversibly targeted, and we can use the Spindly truncation mutants to determine which domains of Spindly are required for the arrest.

Our work further illuminated the interactions between Spindly and the kinetochore. First of all, we observed an association with the RZZ complex; Spindly was observed able to pull down the ZW10 component in IP experiments and Rod was identified in the MS results, confirming the already proposed interaction of Spindly with this complex (Chan *et al.*, 2009) (Barisic *et al.*, 2010). The RZZ complex is important to mediate the localisation of Spindly at the KT, and the C-terminus of Spindly (which is essential for correct KT targeting) has been found to locate close to Zwilch, ZW10 and the N-terminus of Rod (Varma *et al.*, 2013). Moreover, this domain of Spindly has recently been shown to be farnesylated to allow for kinetochore binding (Holland *et al.*, 2015) (Moudgil *et al.*, 2015); this post-translational modification could be required for the interaction with the RZZ complex as well as with the two kinetochore proteins, CENP-E and CENP-F, which were identified in our study as novel binding partners of Spindly.

CENP-E and CENP-F are proteins that require farnesylation to allow correct progression through mitosis (Ashar *et al.*, 2000) (Hussein, 2002) (Schafer-Hales *et al.*, 2007), hence these three fibrous corona proteins, crucial for correct microtubule attachments, are all farnesylated to either interact with each other or with some common farnesyl-binding anchor. Curiously, the interactions with CENP-E and/or -F are insufficient to anchor Spindly at KTs. In the absence of RZZ, CENP-E and -F are present, but Spindly does not reach kinetochores (Barisic and Geley, 2011). Spindly

could therefore require an initial interaction with RZZ to then bind to these CENP proteins and/or a common anchor. FRET experiments could help to elucidate how these kinetochore complexes are interacting with Spindly in the presence and absence of MT attachments. Studies of the dynamics of Spindly in the absence of CENP-E/F can also determine whether these proteins mediate the anchoring of Spindly to kinetochores.

## 6.2 Spindly in interphase

Previous reports on Spindly have looked at it mainly as a mitotic protein, localised within the nucleus before entry into mitosis and then degraded upon exit from mitosis (see **Chapter 1- Paragraph 1.3**). Our aim in this thesis was therefore to untangle the function of human Spindly in non-mitotic cells and more precisely its role in interphase in relation with the cytoskeleton network.

### 6.2.1 Spindly in cell migration

In this work, a novel role for human Spindly in interphase was described, in line with what was already proposed for *D.mel.* Spindly. We identified a pool of the protein within the cytoplasm and on chromatin (see **Fig. 5.1**) and we reported presence of GFP-Spindly at the plus-ends of growing microtubules (see **Fig. 5.2**), validating former data obtained in a different organism (Griffis *et al.*, 2007). Moreover, size-exclusion chromatography analysis of asynchronous cells revealed a fractionation profile that suggested the formation of a complex between Spindly and dynactin not exclusive for mitosis and consequently indicates a possible role for Spindly as an adaptor of the motor complex for different cellular processes (see **Chapter 3**).

Dynein and dynactin have been shown to be active players in the cell migration process; they have been demonstrated to be involved in cytoskeleton reorganisation upon monolayer wounding and in directed cell movement (Palazzo *et al.*, 2001) (Faulkner *et al.*, 2000) (Dujardin *et al.*, 2003). Dynein has also been proved to be crucial for anchoring microtubules at the cell front (precisely at the cell cortex) to then generate forces needed for centrosome repositioning to direct cell migration (Dujardin *et al.*, 2003) (Manneville *et al.*, 2010).

In this thesis, we analysed cell migration in Spindly-depleted cells, revealing slow rates of cell movement in cell-culture based wound-healing assays. We demonstrated



the tight dependency of this phenotype on Spindly expression, since a GFP-Spindly construct could rescue the migration rate up to control levels and independently from mitosis.

We reported an enrichment of hSpindly at the leading edge of fibroblasts and, more precisely, a co-localisation with Zyxin at focal adhesion sites within lamellipodia. Spindly was observed at focal adhesions significantly after Zyxin redistribution had begun, consistent with a role in later stages of cell migration (potentially in relation to focal adhesions maturation or turnover). The similar defects were registered in dynactin depleted cells with a comparable localisation within the lamellipodia in migrating fibroblasts, let us hypothesise a non-mitotic function of Spindly mediated by an association with the dynein/dynactin motor complex, potentially using a similar mechanism as in mitosis.

To validate our hypothesis of a close relation between Spindly and dynein/dynactin in cell migration, it would be useful to look carefully at the two main functions of dynein in this process: cellular trafficking and centrosome positioning. A preliminary analysis was conducted to examine MTOC re-organisation upon wounding in Spindly-depleted cells, but no obvious defects were observed suggesting that Spindly is not crucial for this process to occur. Live imaging experiments tracking centrosome positioning in Spindly-depleted cells would help to rule out this supposition and so to determine if the process happens with the same timing in cells lacking Spindly. On the other side, Spindly could also be involved in the dynein-mediated membrane trafficking process (Schmoranzer *et al.*, 2003) (Manneville *et al.*, 2010). Following this idea, it could be that Spindly plays a role in cell migration similar to the one we showed it plays in mitosis, as a modulator of the targeting and/or processivity of the dynein/dynactin motor complex in order to promote streaming of cargos to the delivery site. Thus,

Spindly could work with dynein/dynactin in different cell cycle phases as a cargo-specific adaptor of the motor complex in different cellular processes.

Future studies will investigate this possibility, looking closely at membrane trafficking in Spindly-depleted cells. Nevertheless, we think that Spindly is not involved with all interphase functions of the dynein/dynactin complex; in fact, lack of Spindly expression does not affect dynein/dynactin-dependent endosome positioning in S2 cells (Griffis *et al.*, 2007) or the morphology of the Golgi apparatus in HeLa cells (data not shown).

The presence of Spindly at focal adhesion sites (within lamellipodia) and its capacity to bind to other proteins important for focal adhesions and/or lamellipodia formation, together with its essential requirement for cell migration, strongly suggest that Spindly is involved with cytoskeletal activity at the leading edge. Future work will be needed to establish if Spindly is directly involved in maturation/turnover of focal adhesions and/or if it can act more as a cross-linker between microtubules and actin filaments near the membrane. To make progress towards this goal, a better understanding of binding partners of Spindly in this molecular process is crucial. In our work we started analysing the network of proteins to which Spindly could be associating to promote cell migration, we gained different interesting insights that would be interesting to study further in the future.

Data collected in this thesis did not allow us to determine the specific pathway in which Spindly acts; however, they did reveal a novel function for Spindly in interphase that surely will need to be deeply explored in the future. It would be interesting to define whether the role of Spindly in this cellular process is solely through dynein/dynactin or whether it has other functions on its own. Both possibilities are plausible and not exclusive; Spindly could, on one side, regulate the motor complex at

the cell front and/or at focal adhesions, and, on the other side, it could interact with actin and/or microtubule filaments and mediate protrusion formation or dynamics.

### 6.3 Final remarks

Most tumours arise from aneuploidy due to chromosome mis-segregation or over-replication and so show chromosomal instability (CIN). CIN can originate from errors during cell division that lead to the total gain/loss or fractionation of chromosomes; CIN occurs often from deletions, amplifications or translocations that arise from breaks in the DNA (Geigl *et al.*, 2008). Over the years it has been shown that SAC impairment is a major cause of CIN; this impairment has been registered with high frequency in many cancer cells and it is associated with either deletions/mutations/truncations within SAC genes (Weaver and Cleveland, 2005). To date, many SAC components have been described playing a role in aneuploidy generation and consequently in carcinogenesis (Barbosa *et al.*, 2011). For example, inherited mutations in the BUB1B gene result in chromosomal mis-segregation and increased tumour incidence in humans (Hanks *et al.*, 2004). Additionally, Mad2 knock out has been widely proven to lead to premature anaphase onset, with subsequent mitotic catastrophe and apoptosis, while the down regulation of Mad1 is associated respectively with SAC inactivation and aneuploidy (Wang *et al.*, 2010) (Bargiela-Iparraguirre *et al.*, 2014) (Kienitz *et al.*, 2005). Similar behaviour has been reported for Bub1 and Mps1 where either down regulation or overexpression is associated with weakening of the SAC and tumour formation (Jeganathan *et al.*, 2007) (Ling *et al.*, 2014). Thus, a debilitated SAC contributes to oncogenesis in many types of cancer confirming that the expression levels of SAC components is crucial to ensure correct cell division (Barbosa *et al.*, 2011).

Intriguingly, the role of dynein has been recently elucidated in some tumours too. Precisely, studies on the light chain LC7 (km23-1) showed that the overexpression of this subunit causes accumulation of cells in prometaphase, retention of BubR1 at KTs and in turn suppression of cell growth, suggesting that high levels of the protein disrupt its functioning in SAC proteins streaming and putting LC7 as a critical cell growth

regulator (Pulipati *et al.*, 2011). Targeting the SAC and chromosome alignment simultaneously has been reported able to kill tumour cells (Janssen *et al.*, 2009). Specific targeting of mitotic- dynein/dynactin can therefore effectively inhibit growth of rapidly dividing cancer cells, and a better understanding of its regulation can identify new targets that can be exploited to inhibit tumour growth.

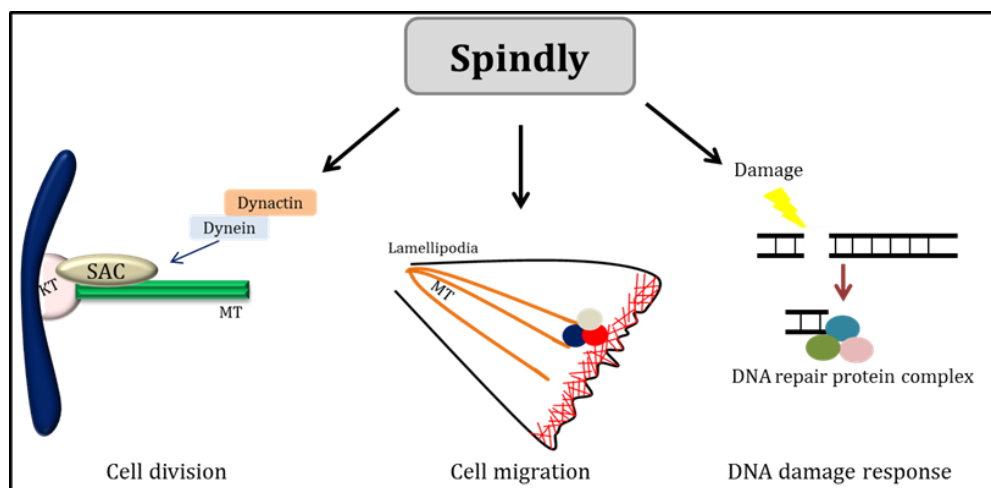
In this thesis we identified new interactions that can show how Spindly is involved in the regulation of the spindle assembly checkpoint and therefore in the transition between metaphase and anaphase during mitosis. Mapping its capacity to interact with the dynein/dynactin motor complex and its association with some of the SAC components, we shed light on novel mechanisms that, upon further studies, would allow us to much better dissect the key inflection point that lies at the heart of the cell division process. Our findings propose Spindly as a multifunctional protein in mitosis; it indeed is involved in the SAC pathway (where it could be an active player of the signalling), in the dynein/dynactin stripping process (where it is critical for timing the silencing of the SAC) and in the ‘sensing’ of correct KT-MT associations (where, interacting with CENP-E, could behave as a coordinator between the SAC signalling and the KT-MT attachments). For all the different functions that it plays, it could be essential that it is not mutated or over/under-expressed as this would deeply alter cell division and potentially induce carcinogenesis. However, to date there is not much known about Spindly and cancer generation (information obtained from [www.oncomine.org](http://www.oncomine.org)), but its critical role in cell division indicates that understanding its mechanism of action could represent an important target for future cancer drug development.

In the present work, we depicted a new role for Spindly in cell migration. Surely this function has to be explored in detail, but cell migration is core to the process of metastasis and many factors that regulate cell migration have been shown to be mutated and/or upregulated in metastatic cells. Actin, actin-regulatory proteins, adhesion molecules and membrane signalling proteins are often overexpressed in solid tumours being involved either in the formation of protrusions (like lamellipodia or invadopodia) or in setting the direction of motility (Stevenson *et al.*, 2012). Researchers are therefore trying to identify pathways that are activated in the processes of metastasis and invasions. So, also in this case, further exploring the role of Spindly in cell migration, including identifying the specific molecular pathways that are disrupted in Spindly depleted cells, the cell types and the type of migration (1D, 2D or 3D) that are affected and defining its binding partners would help to understand how the machinery works. The fact that Spindly localises at lamellipodia suggests that it could be involved in their regulation and dynamics, important process also in cancer cells. Of note is that BubR1 and Mad2 have recently been reported to play a second role in cancer cell migration; their inhibition was shown to slow down the migration process, while their overexpression could promote migration and invasion (Bargiela-Iparraguirre *et al.*, 2014). Thus, it could be that there are other ways in which SAC activity affects the capacity of cancer cells to migrate and to invade surrounding areas. We already demonstrated defects in cell migration velocities upon depletion of Spindly, and so it would be interesting to determine whether over/under-expression of Spindly in cancer cells could affect migration and invasiveness as well.

Finally, we have recently also discovered a further role for Spindly in interphase cells that we think is related with the DNA damage response. We found that Spindly is recruited at double-strand breaks (DSBs) sites upon damage (UV-A irradiation upon Bromodeoxyuridine (BrdU) sensitisation) and that Spindly-depleted cells are more sensitive to DNA damaging agent treatment (preliminary data, not shown). As reported in **Chapter 5**, when we analyse the expression of Spindly in different subcellular compartments, we found a pool of the protein on chromatin, consistent with the possibility that it has an undiscovered function in processes associated with chromatin. We already tested the possibility that this role is dynein-dependent too, but we did not observe the recruitment of dynein at sites of damage. Of note is that a recent paper has pointed at microtubule protein trafficking to the site of damage as the strategy used by microtubule-targeting agents to delay DNA damage repair response in cancer treatments (Poruchynsky *et al.*, 2015). The proteins involved in the DNA damage response tested in the aforementioned publication were demonstrated to interact with dynein, proposing an important role for the motor in this process (Poruchynsky *et al.*, 2015). Thus, it could be that Spindly helps dynein/dynactin to drive the trafficking of DNA repair proteins towards the damaged sites, but then Spindly can localise at sites of DNA DSBs as well. In our analyses, we also recorded recruitment at DSBs of Bub1 and BubR1 (preliminary data, not shown), in line with a recent report that has shown involvement of Bub1 in non-homologous end joining repair pathway in human cells (Jessulat *et al.*, 2015). Many reports have also highlighted the role of phosphatases in the DNA damage response (Freeman and Monteiro, 2010). So, our working hypothesis is that Spindly is bound by or recruits BubR1 (and/or Bub1) at sites of damage, and, once there, it helps to resolve the damage, potentially by recruiting phosphatases. Data collected so far represent a good starting point to move further in studying the roles of Spindly in DNA damage-repair and understanding how mitotic signalling cascades could be repurposed

to guard the genome during interphase. We know that defects in DNA damage response induce genomic instability and eventually carcinogenesis (Hosoya and Miyagawa, 2014); once again alterations in Spindly function could play a role in the cancer generation process. Defects in its expression could impair the DNA damage response and consequently allow tumour formation.

Overall, Spindly, as an extended (70nm) protein with distinct binding surfaces, could behave as a scaffold protein involved in the assembly of several large molecular machines where different proteins have to be brought together to orchestrate a particular cellular activity. The finding that Spindly is fundamental in several cellular processes let us to hypothesise that its expression, localisation, and post-translational modifications have to be carefully modulated to promote all of its varied functions (Fig. 6.1). Therefore, it is plausible that, if mis-regulated, Spindly leads to generation of defective cell divisions, altered cell migration and inefficient DNA damage repair, pathways that, when inhibited or altered, precede tumorigenesis.



**Figure 6. 1.**Overall diagram summarising the different functions of human Spindly.



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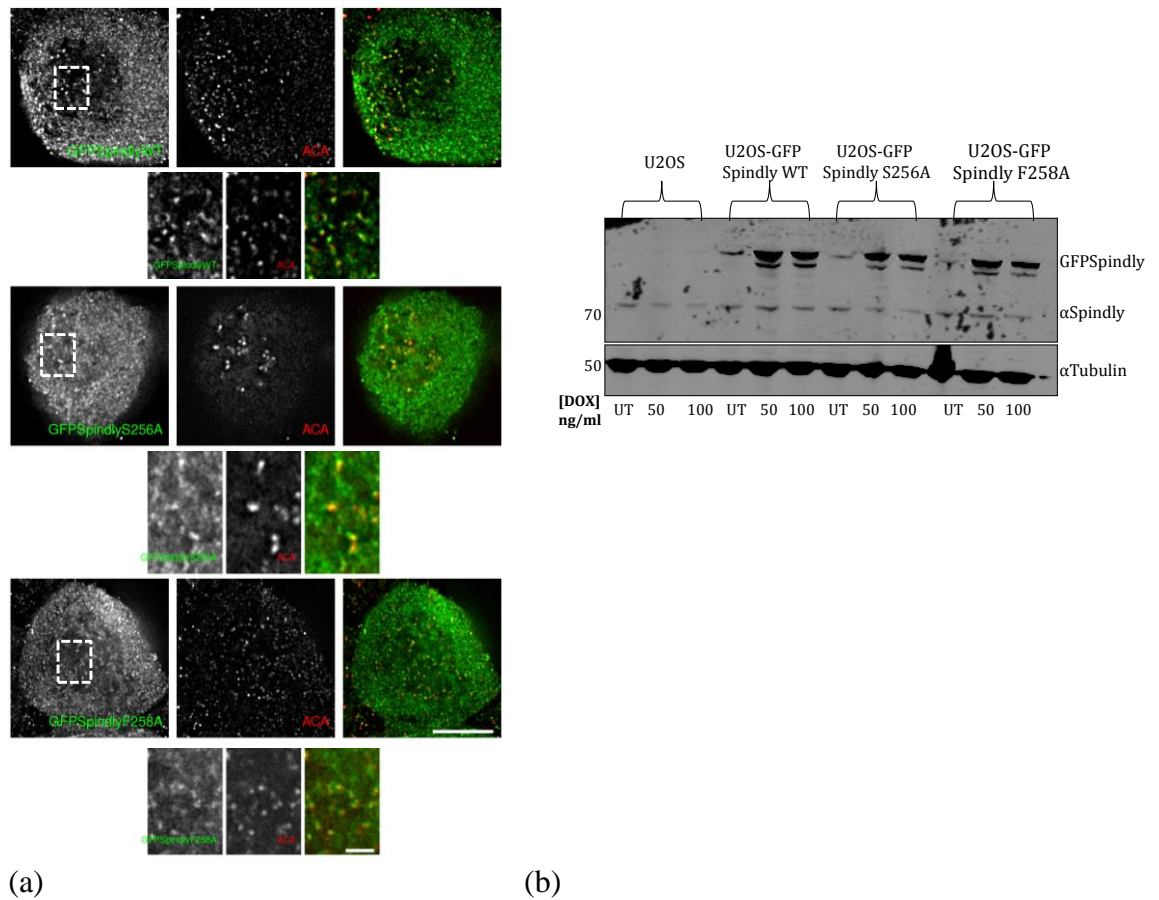
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## **8 . Appendix**

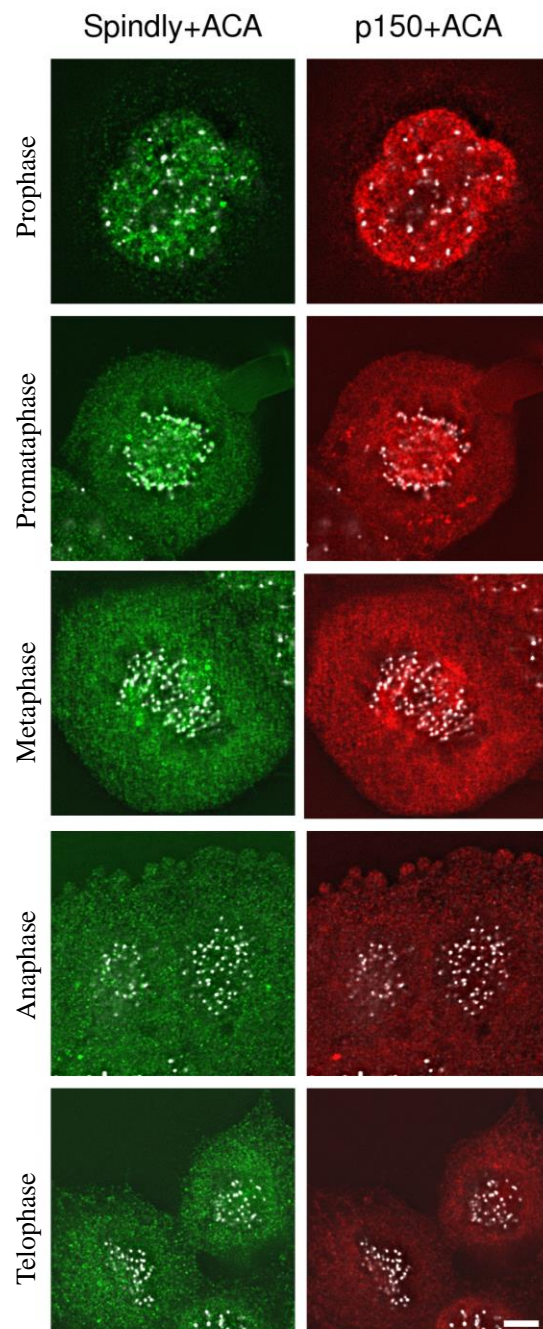
## 8.1 Generation of stable cell lines expressing GFP Spindly



**Figure 8. 1. U2OS cells express correctly GFP Spindly.**

U2OS cells stably expressing GFP Spindly WT, S256A mutant or F258A mutant. (a) Fluorescence images showing correct kinetochore localisation of all three constructs expressing GFP Spindly (green), co-localising with the kinetochore marker ACA (red). Details underneath each single GFP panel show correct KT localisation of GFP Spindly constructs (dashed white box). Scale bar 10  $\mu\text{m}$  (or 2  $\mu\text{m}$  for details). (b) Western blot experiment of U2OS cells expressing GFP Spindly constructs upon administration of Doxycycline (either 50 or 100 ng/mL for 24 hours). Tubulin was used as loading control.

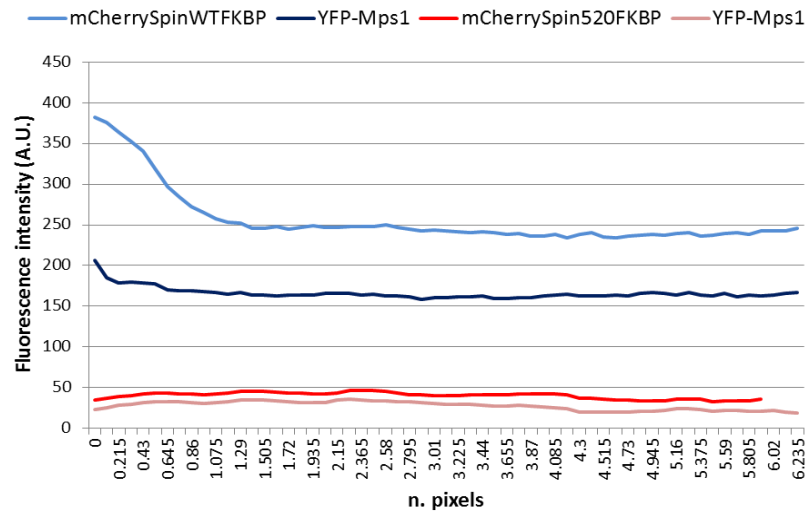
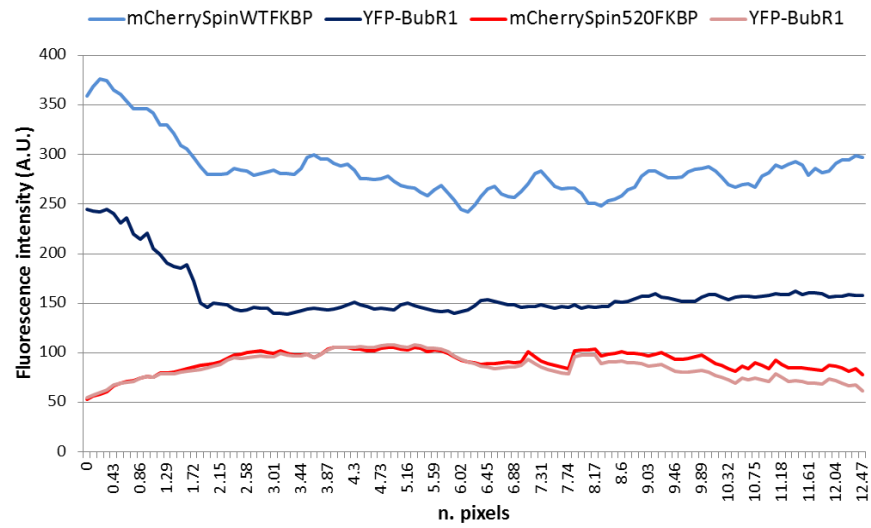
## 8.2 Localisation of Spindly in mitosis

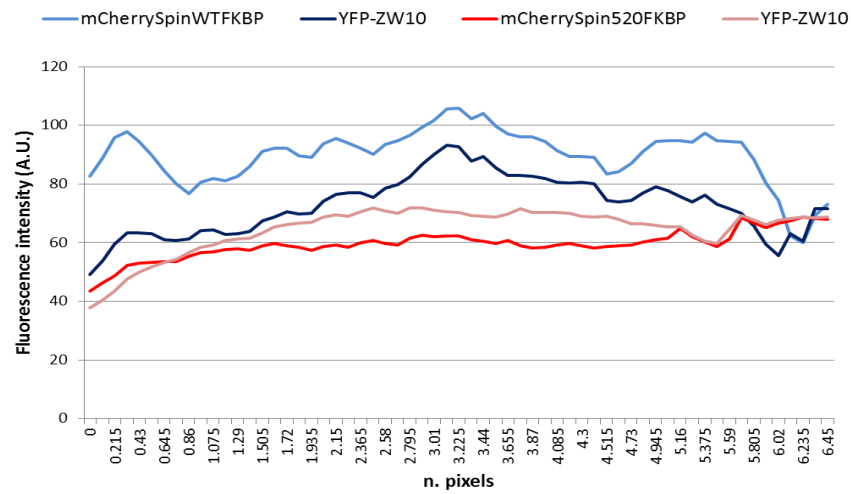
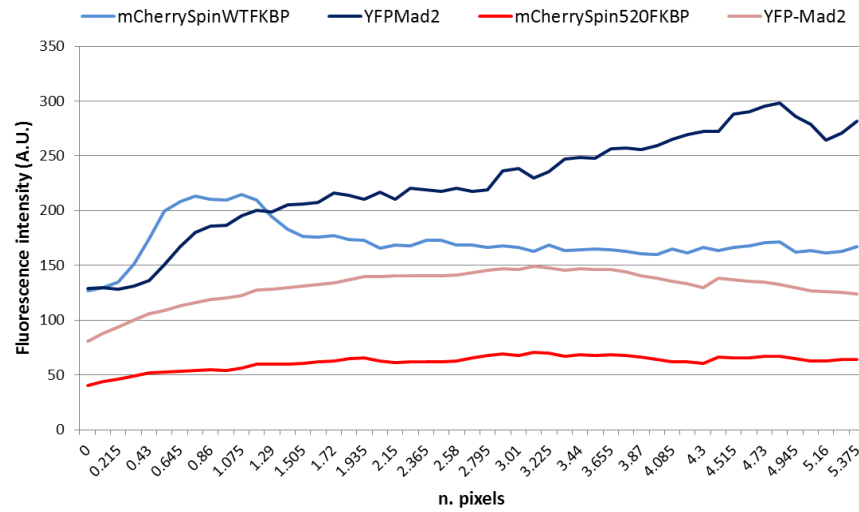


**Figure 8. 2. Spindly localises at kinetochores in mitosis.**

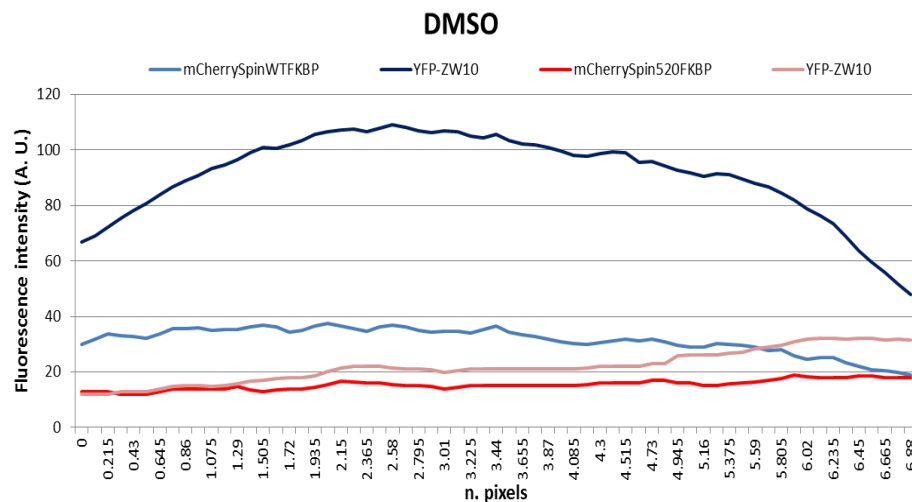
U2OS cells were stained with anti-Spindly (green) or anti-p150 (red) and anti-ACA (kinetochore marker, white) antibodies. Spindly and p150 co-localise with ACA at KT's mainly during prometaphase. Scale bar 5  $\mu$ m.

### 8.3 Rapamycin mis-localisation experiment measurments





(b)

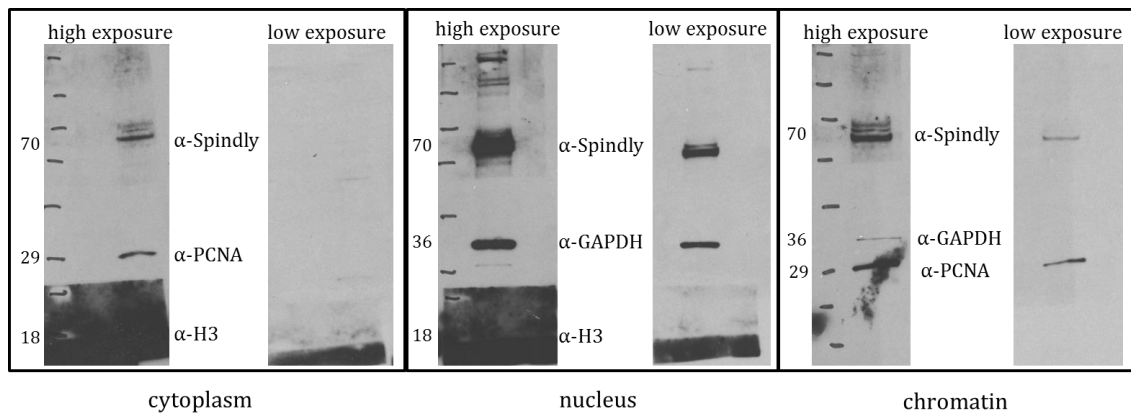


**Figure 8. 3. Expression of Spindly 1-520 does not affect interaction with SAC components.** HeLa cells transfected with mCherry-SpindlyFKBP WT or 1-520, Lin11FRB, YFP-SAC component tested. Images were collected upon staining under fluorescence microscopy (see Chapter 4, Fig. 4.6 and 4.7) and measurements were carried out by drawing a line between the two edges of the cell (n= 30). Comparison between



expressions of mCherrySpinWT-FKBP or mCherrySpin520-FKBP reveal similar capacities of the two constructs to interact with the different SAC component tested upon administration of Rapamycin (4 $\mu$ M) (a). In (b) is shown representative values obtained upon administration of DMSO. Here is reported the graph obtained for YFP-ZW10 to confirm lack of recruitment without addition of Rapamycin (for the other proteins tested similar negative results were obtained, data not shown).

## 8.4 Spindly is not only a nuclear protein



**Figure 8. 4. Spindly is not exclusively a nuclear protein.**

Fractionation experiment of U2OS cell lysate performed to analyse the expression of Spindly in different cellular compartments. Here is reported a western blotting analysis to cross check the isolation of the different fractions; positive isolation is reported in chapter 5, fig. 5.1. Antibodies tested as indicated. Anti-PCNA was used as nuclear marker; anti-H3 (anti-histone 3) was used as chromatin marker; anti-GAPDH was used as cytoplasm marker. This result suggests the presence of Spindly in all the fractions; however validation of the fractions with more stringent markers would be needed.

## 8.5 Mass spectrometry analysis of Spindly interacting proteins in mitosis

Protein IDs	Peptides	Q-value	Noc IP Sum	Noc IgG Sum	STLC IP Sum	STLC IgG Sum	UT IP Sum	UT IgG Sum
sp Q14204 DYHC1_HUMAN;tr H0YJ21	215	0	528168900	92346940	257703500	159281070	781034600	135430070
sp Q09666 AHNK_HUMAN;tr Q6ZQN2	201	0	433201460	79022340	169055240	96483000	1278503200	54104830
sp P78527 PRKDC_HUMAN;sp P78527-	163	0	330012800	43917790	165526680	54963120	1067001600	76804910
sp P15924 DESP_HUMAN;sp P15924-3	126	0	324473000	103972000	127217000	123059000	131292000	105272400
sp P21333-2 FLNA_HUMAN;sp P21333	111	0	243425630	72122500	140002020	71577750	184456790	79397900
sp P49327 FAS_HUMAN;tr Q6PJJ3 Q6I	107	0	301770100	51971460	184568742	90015300	605831550	51773500
tr AOA024R1N1 AOA024R1N1_HUMAN;	106	0	132215096	50180010	143137050	29035765	1258123900	87227694
sp Q9Y490 TLN1_HUMAN;sp Q9Y4G6	102	0	102428289	24554119	70755350	53836396	569220070	42596308
sp Q15149-7 PLEC_HUMAN;sp Q15149	102	0	83572302	7845233	41085310	9090485	61530530	8300600
tr E1NZA1 E1NZA1_HUMAN;sp Q9261	91	0	81628000	9560500	40015170	12054000	172988900	9482870
sp P35580 MYH10_HUMAN;sp P35580-	91	0	69568510	23748000	71454000	20400607	512159725	53693120
sp Q14008 CKAP5_HUMAN;sp Q14008-	83	0	157530714	37099830	86857620	32304410	193533550	10886000
tr AOA087X1U6 AOA087X1U6_HUMAN;	81	0	137759700	21027380	44927790	35799026	210681900	26783630
sp P49454 CENPF_HUMAN;tr AOA087V	81	0	81830590	0	16028542	0	12206621	0
sp P58107 EPIPL_HUMAN	79	0	3357400	353460	1422300	217940	822750	417090
sp Q6P2Q9 PRP8_HUMAN;tr I3LQJ9 I3	78	0	22920500	4948800	27457217	5309803	150393300	9645700
sp Q00610-2 CLH1_HUMAN;sp Q00610	76	0	205622100	27204497	92999640	51295644	299444370	21604020
sp O75643 U520_HUMAN;tr A4FU77 A	76	0	68292700	12518000	45539683	13953000	284230650	14433412
tr F8VPD4 F8VPD4_HUMAN;sp P2770	71	0	117154520	12823000	72360500	22257000	315567560	21177300
sp P35908 K22E_HUMAN	71	0	34077040	14776100	12355800	34741100	22306600	17050200
sp P04259 K2C6B_HUMAN	61	0	48265500	23237400	19150300	24509000	18913200	21980400
tr AOA024R9Y3 AOA024R9Y3_HUMAN;t	61	0	25657000	2316560	9387320	2818400	53810910	5241688
sp P07814 SYEP_HUMAN;tr Q3KQZ8 C	59	0	93166230	20675909	39208200	22423869	257429600	36766460
tr B2R853 B2R853_HUMAN;tr B4DXK4	59	0	10805070	7390400	4767110	11709100	8129400	10436300
sp O75369-2 FLNB_HUMAN;sp O75369	58	0	12650777	2964792	6294369	2204756	7520170	5800400
sp P13639 EF2_HUMAN;tr Q8TA90 Q8	54	0	443794680	136084700	304680760	130504870	982477900	251745000
tr AOA024R3T8 AOA024R3T8_HUMAN;s	54	0	163483500	54091264	106764390	40571410	537016190	90618890
sp P55060-3 XPO2_HUMAN;sp P55060	51	0	381581980	64661091	164130100	55287740	661848660	40056970
tr AOA024R652 AOA024R652_HUMAN;s	51	0	301874690	101742710	253822400	77069210	1133105000	123454640
tr E7EUU4 E7EUU4_HUMAN;sp Q0463	51	0	135607135	27276960	71118470	29886214	348068350	28149686
sp Q04637-3 IF4G1_HUMAN;tr B4DGF	51	1	1854300	0	1168800	242520	2807146	415780
tr AOA024R1Y2 AOA024R1Y2_HUMAN;s	50	0	146042690	17710920	49655030	11282190	216243700	28301667
sp Q9Y520-4 PRC2C_HUMAN;tr E7EPN	50	0	32112000	5362849	17583120	4589100	93520530	3654400
sp P41252 SYIC_HUMAN;tr Q59G75 Q	49	0	92430900	17332520	44353457	18403069	269842530	40242739
tr F1TQJ2 F1TQJ2_HUMAN;sp Q14789	49	0	13878910	5623097	15755060	6749400	10764800	1809210
sp Q08211 DHX9_HUMAN;tr Q58F26 C	48	0	74236530	12533300	42501771	11185740	351794940	27509328
tr V9HWB8 V9HWB8_HUMAN;sp P146	46	0	440740300	123027100	788513300	160118700	1568318000	220544500
sp P22102 PUR2_HUMAN;tr Q59HH3 C	46	0	153532578	35647160	95177720	40936600	477814000	56037900
tr B2RCM2 B2RCM2_HUMAN;sp Q9P2	46	0	75910570	16585900	56500470	18656380	354385000	56680130
sp P51610-4 HCF1_HUMAN;tr A6NEN	46	0	62500000	16861680	77459800	15613833	104375200	12549950
sp O75533 SF3B1_HUMAN;tr Q7Z497	46	0	56275180	9187000	25002340	5597179	133598799	9941834
tr V9HW29 V9HW29_HUMAN;sp P331	45	0	47679000	10077220	28609340	6492103	118534030	11967585
sp P49792 RBP2_HUMAN;tr D3DX73 C	45	0	24866210	2011090	10220030	3021100	8347660	763398
sp ASYKK6 CNOT1_HUMAN;sp ASYKK6	45	0	23120000	3630200	13555380	4846814	104104000	3318000
tr F5GWX5 F5GWX5_HUMAN;sp Q148	45	0	20865130	3053900	17571300	2193600	92144947	3824200
sp Q9NUJ2 MDN1_HUMAN;tr Q6AI22	45	0	12782000	564690	4084200	797000	33351611	1050700
sp P51610 HCF1_HUMAN;sp P51610-	45	0.000358	3552100	1233400	3734700	1056100	5837500	1211310
tr AOA024RDY0 AOA024RDY0_HUMAN;	44	0	115950500	19952620	65231200	17074910	262382100	23328860
tr AOA024R4E5 AOA024R4E5_HUMAN;s	44	0	63558300	17718519	46996680	19273982	329368400	19804420
sp P48634 PRC2A_HUMAN;sp P48634-	44	0	60149000	1380000	31129000	1643400	164703950	3213400
tr Q24JU4 Q24JU4_HUMAN;tr J9R021	44	0	47399700	6749340	20964880	7476500	94518659	11075630
sp Q5VYK3 ECM29_HUMAN;tr J3KN16	44	0	41104000	3111400	12021620	4271900	82322700	4941604
sp Q16531 DDB1_HUMAN;tr F5GY55 F	44	0	31155350	6159100	15372550	6792300	51495000	9188693
sp Q7KZF4 SND1_HUMAN;tr B2R5U1	43	0	90937400	23458989	56565938	18983180	108176700	39066390
sp P52732 KIF11_HUMAN;tr B2RAM6	43	0	62652408	11175000	53239209	9320800	168457610	9489800
tr C1PHA2 C1PHA2_HUMAN;tr M1V48	43	1	0	1293056	0	1062400	0	0
sp P12270 TPR_HUMAN;tr Q15624 Q1	42	0	22054360	83693	14641420	0	23072000	1608480
sp Q9BQGO MBB1A_HUMAN;sp Q9BQGO	42	0	14782000	343160	3779900	0	100699494	1050600
sp Q5T457-3 UBR4_HUMAN;sp Q5T457-	42	0	5600200	0	3521960	518950	27864000	562960
sp O75694-2 NU155_HUMAN;tr B4DLT	41	0	130305800	24210890	76356300	21939390	253052990	42336507
sp P11717 MPRI_HUMAN;tr Q59E23 C	41	0	24668000	79314	12890700	282960	5908100	133580
sp P29144 TPP2_HUMAN;tr Q5VZU9 C	41	0	24567900	3220800	15131000	2705400	106461130	7570720
sp Q93008-1 USP9X_HUMAN;sp Q93008-	41	0	17866000	2226619	13182630	2361200	60696490	2633440
tr AOA024RD80 AOA024RD80_HUMAN;	40	0	356569620	168655300	569651500	184666500	671376600	240059000
tr A8K8U1 A8K8U1_HUMAN;sp Q86VP	40	0	84462600	13931369	60075420	17472660	272799480	24726920
sp Q709C8-3 VP13C_HUMAN;sp Q709C8-	40	0	42056300	1157274	9339710	139220	57476790	0
sp Q15643 TRIPB_HUMAN;tr AOA087V	40	0	14119000	4376680	12172630	2777200	6966480	3323110
sp O14980 XPO1_HUMAN;tr B7ZB16 E	39	0	123485900	22168830	55852380	21393700	319223900	34266830
tr K9JA46 K9JA46_HUMAN;sp P07900	39	0	103072290	62687048	170329110	60250938	154359300	68759300
tr B3KMX0 B3KMX0_HUMAN;tr AOA024R	39	0	48796860	8956466	31155300	7117600	191082520	16143259
sp P53621 COPA_HUMAN;sp P53621-2	39	0	28630320	2630800	14939928	3310000	48105000	3961140
tr Q86VX4 Q86VX4_HUMAN;tr B0AZQ	39	0	20131000	5461700	10641000	2433400	65723100	3984210
sp P52701 MSH6_HUMAN;sp P52701-3	39	0	19806000	3404098	4545850	1987200	78852760	5049900
tr A8K5IO A8K5IO_HUMAN;sp P08107	38	0	320189900	197561300	430216600	183972200	162203300	93576300

tr Q53HJ4 Q53HJ4_HUMAN;tr B2R7C5	38	0	52707500	11337673	36234950	6738050	159714010	18625730
tr G1UI16 G1UI16_HUMAN;sp Q29RF7	38	0	20000420	3064900	11327150	3150300	79761013	3122000
sp Q92621 NU205_HUMAN;tr B4DE72	38	0	19255430	7545191	25398000	3905431	104344800	8208210
tr A0A024RAM4 A0A024RAM4_HUMAN	38	0	9082000	1314600	6973230	671730	69037690	4971550
sp Q13813-3 SPTN1_HUMAN;sp Q1381	38	0	4654800	0	1946500	0	477250	530370
sp P07197 NFM_HUMAN;tr E7ESP9 E7	37	0	166559000	12007200	80508000	6580200	12667000	1002220
tr V9HW96 V9HW96_HUMAN;sp P783	37	0	139182147	60569550	355170020	73806130	433010100	111062300
sp P49588 SYAC_HUMAN;sp P49588-2	37	0	57332700	16672010	35006377	13413310	148381382	36538470
sp Q99460 PSMD1_HUMAN;sp Q99460	37	0	56263670	14620000	26611690	13795020	135785910	25670180
tr A0A024RCN6 A0A024RCN6_HUMAN	37	0	40397840	8273500	29976177	9193234	144519130	12525000
sp P50851 LRBA_HUMAN	37	0	9244800	1435980	7312770	2030400	42369000	2247500
sp P50851-2 LRBA_HUMAN;tr E9PEM5	37	0.006311	454740	0	449460	0	1742100	0
tr A0A024R1N4 A0A024R1N4_HUMAN	36	0	127645502	70031460	205347383	45201420	284589200	50559300
tr A3RJH1 A3RJH1_HUMAN;sp Q92499	36	0	78446370	38155971	140453070	29992000	124270320	31241060
sp Q95613-2 PCNT_HUMAN;sp Q95613	36	0	15200000	0	6738100	0	6929300	0
sp A6NHR9-2 SMHD1_HUMAN;sp A6N	36	0	8115190	792119	5496405	892984	54166610	2026700
sp P35658-2 NU214_HUMAN;sp P3565	36	0	7692300	1375600	9707900	1298700	88374039	4232300
tr V9HWE1 V9HWE1_HUMAN;sp P086	35	0	103134100	8396900	78299070	3145630	4966950	58616
tr V9HW37 V9HW37_HUMAN;sp P486	35	0	78039400	19427200	136065560	29672470	225254670	44264450
sp Q14683 SMC1A_HUMAN;sp G8ILG1	35	0	42202000	4566723	11185560	9892510	90684300	12356780
sp Q15029-2 U5S1_HUMAN;tr Q61BM8	35	0	26614000	4097700	17405880	2264700	97873190	7228700
sp Q02224-3 CENPE_HUMAN;tr A0A08	35	0	16080000	0	4829500	0	12052000	218510
tr Q5CAQ5 Q5CAQ5_HUMAN;tr V9HW	35	0	10475410	7338680	8938820	4415490	14888040	11454300
sp Q8N3C0 ASCC3_HUMAN;tr B4DR60	35	0	6667300	0	4974760	394120	31978100	0
sp P50990 TCPQ_HUMAN;sp P50990-2	34	0	123239940	30712900	219449350	47037240	321011200	63834600
tr A0A024R4F1 A0A024R4F1_HUMAN;s	34	0	76416100	55742200	58238500	36283800	54468700	120410000
sp P22314-2 UBA1_HUMAN;tr A0A024	34	0	56543704	26457400	32519560	17250300	106883950	53134900
tr V9HW22 V9HW22_HUMAN;sp P111	34	0	50618760	25164960	56264280	26229360	66583800	24357400
tr Q4W4Y1 Q4W4Y1_HUMAN	34	0	37270500	10449000	31316300	10606831	113633100	21493080
sp Q6P2E9 EDC4_HUMAN;sp Q6P2E9-	34	0	34733000	3964500	19300000	9167000	45857000	2726022
tr A8K6X3 A8K6X3_HUMAN;sp Q7L014	34	0	31076780	3300800	21547257	2402908	102114600	3801022
sp Q9Y5B9 SP16H_HUMAN;tr Q0VGA3	34	0	29446918	17617670	19926500	18547630	199456150	13495804
tr Q53H50 Q53H50_HUMAN;sp P4789;	34	0	28004400	9607855	45289455	16156490	117408940	18169850
sp Q14203-3 DCTN1_HUMAN;sp Q142	34	0	20991000	3781417	14370024	8994400	45904270	6210100
tr A0A024R7L5 A0A024R7L5_HUMAN;s	34	0	16979100	4319307	17861400	3265490	65170700	6116477
tr B7ZMF2 B7ZMF2_HUMAN;sp Q9NVI	34	0	12003410	353930	4011100	336800	30015000	204240
sp P55196 AFAD_HUMAN;tr J3KN01 J	34	0	11389789	1102255	8922007	2141576	53719756	1846127
sp Q13428-8 TCOF_HUMAN;sp Q13428	34	0	1831200	675600	1251000	455800	35948480	3553195
sp Q8WUM4 PDC6I_HUMAN;sp Q8WU	34	1	1078600	0	0	0	2643400	0
tr B3KX11 B3KX11_HUMAN;sp P49368	33	0	15625280	38147550	220600460	50524760	309240600	58841400
tr A0A024R1X8 A0A024R1X8_HUMAN;s	33	0	143388000	54668000	61797000	66071000	71139000	51665700
sp P13010 XRCC5_HUMAN;tr Q53T09	33	0	117265320	52825270	164008680	43670240	277893900	63528000
sp Q00839 HNRPU_HUMAN;tr B3KX72	33	0	115841000	25776120	62048670	11747480	442974100	42853090
sp Q13263 TIF1B_HUMAN;tr B2R8R5 E	33	0	115522100	31030310	78792470	30337340	330238110	38371050
tr X5D2J9 X5D2J9_HUMAN;sp P78347-	33	0	44949900	11211000	29074180	8629084	141347846	17458325
tr A8K492 A8K492_HUMAN;sp P56192	33	0	42469970	6048800	20548500	3788800	193133800	15033770
sp Q9NR30 DDX21_HUMAN;sp Q9NR3	33	0	25568900	6999690	20607949	3530790	144546294	8576110
tr B1AHB0 B1AHB0_HUMAN;sp P3399;	33	0	16515200	3842700	28110300	6956900	96884200	10774490
sp Q14697-2 GANAB_HUMAN;tr V9HV	33	0	10509540	4485705	5894186	3582156	16873118	14941500
sp P52948-6 NUP98_HUMAN;sp P5294	33	0	6255600	1297400	4766674	855920	59205000	2932300
sp Q14974 IMB1_HUMAN;tr B2RBR9 E	32	0	149174685	43261170	91952280	39186695	443427860	54341300
tr Q53HV2 Q53HV2_HUMAN;sp Q9983	32	0	74587336	17170950	159322140	28069400	269826130	36083960
tr B5BTY4 B5BTY4_HUMAN;sp O00571	32	0	52813945	16297316	69045318	16522382	139271390	13677920
sp Q14566 MCM6_HUMAN;tr Q4ZG57	32	0	52051440	13118070	34608800	7422650	164866400	17981980
tr F4ZW66 F4ZW66_HUMAN;sp Q1290	32	0	33547700	10759045	44180270	12184570	271581000	22848510
tr Q53GX7 Q53GX7_HUMAN;sp P2663	32	0	32303966	10808352	74833300	10103377	165069500	24032290
tr A0A024RDG1 A0A024RDG1_HUMAN	32	0	22802000	6652700	21265000	5486800	180654160	15507003
tr H3BPE1 H3BPE1_HUMAN;tr H3BQK5	32	0	2568700	0	1349700	0	21004000	237860
sp P55196-3 AFAD_HUMAN;tr H0Y8U8	32	0	737200	284240	534200	0	0	206460
sp Q9UPN3-3 MACF1_HUMAN;sp Q9U	32	1	0	0	0	0	1045200	0
tr B3KMS0 B3KMS0_HUMAN;sp Q1502	31	0	13254000	1478500	1830000	1839600	53191000	2758003
sp Q15075 EEA1_HUMAN;tr A8K7J3 A	31	0	9696900	520780	2526300	733800	38365000	1739660
tr V9HWF4 V9HWF4_HUMAN;sp P005	30	0	511032500	203926300	860284200	151211700	1453560500	165358900
sp P17987 TCPA_HUMAN;tr E7ERF2 E	30	0	173151540	44137990	322780200	55538910	385475800	74688700
sp P50991 TCPD_HUMAN;tr A8K3C3 A	30	0	133179186	39256280	212914000	48074210	328119560	61018400
tr A8K8N7 A8K8N7_HUMAN;tr A8K9T5	30	0	33313620	5679400	13202000	8633170	142567660	12486504
sp P54136 SYRC_HUMAN;sp P54136-2	30	0	31752063	7973707	53852160	9353820	108638270	9450229
sp P06737-2 PYGL_HUMAN;sp P06737	30	0	27910600	7407679	20592000	8485158	38098490	9340913
sp Q14C86-5 GAPD1_HUMAN;tr A0A0	30	0	23150000	2828660	8766490	5609600	54645120	4975600
tr B2ZZ90 B2ZZ90_HUMAN;sp Q13085	30	0	5056800	422740	3496600	1135600	29415000	1233200
tr A0A0A0MSW3 A0A0A0MSW3_HUM	30	1	0	0	0	0	0	0
sp P29508 SPB3_HUMAN;tr Q5K684 C	29	0	127713440	3338120	7262210	11266630	26023100	5278230
tr V9HWN7 V9HWN7_HUMAN;sp P04	29	0	72576270	16914720	66796400	20708650	59198620	38196700
tr B2RBA6 B2RBA6_HUMAN;sp P33993	29	0	39416900	10847000	66738671	7923190	150251570	19636570
sp Q8TEX9 IPO4_HUMAN;sp Q8TEX9-2	29	0	34357750	4569000	16109060	4220600	55230310	5128380
sp P52272-2 HNRPM_HUMAN;sp P522	29	0	28126063	12403570	36501241	9158744	176694300	24996550

tr A0A024R4A0 A0A024R4A0_HUMAN;	29	0	23841350	14286110	19939360	9204550	111871570	31231300
sp P49736 MCM2_HUMAN;tr B3KXZ4	29	0	20255390	3019600	8352280	1694700	40296200	7868160
tr A8K6V3 A8K6V3_HUMAN;sp Q1539	29	0	16257487	2909469	9048200	574671	81417271	5294900
tr A0A024QZW7 A0A024QZW7_HUMAN	29	0	7598700	440950	2857200	931877	43642000	2329293
tr Q8N516 Q8N516_HUMAN;sp O9516	29	0	7587200	1758260	6649600	2856300	76912990	2448000
tr B2R9K8 B2R9K8_HUMAN;tr A0A024	28	0	115574360	32785690	198874190	39594230	301670300	61973500
tr Q53YD7 Q53YD7_HUMAN;sp P2664	28	0	81412070	44434960	187532970	42321890	334649800	86498700
tr A4D210 A4D210_HUMAN;tr A0A024	28	0	43240010	8116100	21630840	5001431	78415480	11788035
sp P49915 GUAA_HUMAN;sp P49915-2	28	0	27120780	6990800	35125980	7195078	88980880	14390660
sp P31939-2 PUR9_HUMAN;tr V9HWH	28	0	18410847	7037687	23087000	6378875	11801000	5402750
sp P43246 MSH2_HUMAN;sp P43246-2	28	0	16325490	2306700	8846861	843629	64653400	6720500
tr A0A0A0MRM8 A0A0A0MRM8_HUMAN	28	0	9136700	1115375	6135160	1033200	53294000	2821820
tr B2ZZ89 B2ZZ89_HUMAN;sp Q01082	28	0	1813600	123400	3425780	0	0	175330
sp Q96N67-4 DOCK7_HUMAN;sp Q96N	28	0	876640	144180	5739950	0	23163500	1064700
tr Q6IPT9 Q6IPT9_HUMAN;tr Q6IPS9	27	0	1339230500	536482100	2209603000	580905100	2887259000	694210000
sp P43243 MATR3_HUMAN;tr A8MXP9	27	0	98480324	21118980	78085870	22386855	329156700	47107430
tr J3KTA4 J3KTA4_HUMAN;sp P17844	27	0	69398000	21278260	114242480	20378186	185286450	16514330
sp O95373 IPO7_HUMAN;tr B3KQG6 E	27	0	68718250	12421000	37203497	9216800	123750000	14553220
sp Q9Y230 RUVB2_HUMAN;sp Q9Y230	27	0	64999531	25131400	119850920	25552217	253747680	46501510
sp P42166 LAP2A_HUMAN;sp P42167-	27	0	53432700	17236810	121929870	13066980	266613290	28090937
tr B3KTJ9 B3KTJ9_HUMAN;sp Q8N163	27	0	27945000	6048440	18166600	5113800	95722996	7620770
tr V9HWB4 V9HWB4_HUMAN;sp P110	27	0	17328900	6285170	10311090	5996820	28939200	10879400
sp Q08J23 NSUN2_HUMAN;sp Q08J23	27	0	14199480	2135082	7844010	1367200	61438400	2170100
sp Q43143 DHX15_HUMAN;tr B4E0S6	27	0	13857700	5265720	23454340	5924090	25614500	7398660
sp P42285 SKL2L2_HUMAN;tr A8K6I4 A	27	0	12059000	2438080	10623340	3226500	51328350	4850900
sp O00203-3 AP3B1_HUMAN;sp O0020	27	0	9380900	526830	6136940	2025190	76888810	3765628
sp Q8NI27 THOC2_HUMAN;tr B4DKZ6	27	0	7584240	1352890	4444330	1413165	37353160	894660
tr B4DM67 B4DM67_HUMAN	27	1	0	0	0	0	1763700	0
tr V9HWB9 V9HWB9_HUMAN;sp P003	26	0	165579400	159731100	229950000	136657700	480148300	182728500
sp Q02413 DSG1_HUMAN;sp Q02413-2	26	0	65225000	44126000	45136200	57818000	44251000	43756600
sp Q16658 FSCN1_HUMAN;tr B3KTA3	26	0	61479197	20595904	99073836	21997216	210441800	40301560
sp Q92945 FUBP2_HUMAN;tr A0A087V	26	0	36108600	77410027	101472974	102664530	63375000	68464700
sp P63010-3 AP2B1_HUMAN;tr H0UID	26	0	27133000	6724400	17663960	5375600	77681000	4701400
sp Q9BPX3 CND3_HUMAN;tr Q6NUR1	26	0	25444000	2706100	8266000	2067000	57599393	2277600
tr A0A087WZ13 A0A087WZ13_HUMAN	26	0	25358210	11652510	61693000	9062020	92170450	21640710
sp P14868 SYDC_HUMAN;tr D3DP78 C	26	0	24952500	6796000	28038000	6174190	91753080	10383430
sp Q9UPN6 SCAF8_HUMAN;tr B7Z876	26	0	24618550	3441100	9982220	3313200	60413750	1792570
tr Q86VG2 Q86VG2_HUMAN;sp P2324	26	0	23718219	13104810	29646488	8287900	281677930	26808100
tr A8K607 A8K607_HUMAN;sp Q9UIA5	26	0	16223000	2650000	9454710	217736	24324000	2618600
tr V9HW80 V9HW80_HUMAN;sp P550	26	0	12784000	2804100	3806400	1121300	16701500	5744730
sp O94979-3 SC31A_HUMAN;sp O9497	26	0	10756000	1924600	3546100	1217217	34940900	2962798
tr E9PLK3 E9PLK3_HUMAN;tr B7Z899	26	0	9832570	2748400	4154000	748810	850910	3596600
tr Q5K634 Q5K634_HUMAN;tr Q86W0	26	0	6598465	0	0	473720	2583800	0
tr A0A087X0H9 A0A087X0H9_HUMAN;	26	0	5520400	5055300	21419710	4214200	59591010	21366420
tr Q59GJ0 Q59GJ0_HUMAN;sp O43432	26	0	3904200	986640	3522300	697950	19723000	598790
tr B4DP06 B4DP06_HUMAN;tr H7C1S2	26	0	2870200	1354200	5063400	801030	1935900	957800
tr B3KWD0 B3KWD0_HUMAN	26	0	536350	0	0	0	2370600	0
tr B3KPS3 B3KPS3_HUMAN;sp P68363	25	0	852260000	339838100	1722802500	360276100	3385555000	568599000
tr Q8I229 Q8I229_HUMAN;sp P68371	25	0	505369370	151583900	1007816200	196518000	1894110000	292897600
tr A8K7F6 A8K7F6_HUMAN;sp P60842	25	0	254899650	100679450	411240700	95148600	722942500	139249100
sp Q92841 DDX17_HUMAN;tr H3BLZ8	25	0	27830783	9969240	50080118	7686811	143448530	14114590
sp Q9Y678 COPG1_HUMAN;tr B3KW21	25	0	26147000	3949200	13267870	3215380	76485500	7092810
tr V9HW65 V9HW65_HUMAN;tr A0A0	25	0	25402500	13739500	10626600	13773600	41156800	22060000
sp Q13200 PSMD2_HUMAN;tr Q59EG8	25	0	20719000	5018163	12559010	5250680	77079750	16550460
sp Q71U36-2 TBA1A_HUMAN;sp Q71U	25	0	15757890	4436988	37816000	2552310	35931390	7455804
sp Q9H3S7 PTN23_HUMAN;tr B4DJ12	25	0	14969000	805880	4281800	1288700	39603410	960020
sp Q9Y262 EIF3L_HUMAN;tr B0QY89 E	25	0	14479099	1834700	27937940	2051720	38446000	5466551
sp Q96QK1 VPS35_HUMAN;tr Q53FR4	25	0	12423210	5104870	28980132	5041126	52272910	8906291
tr A0A024R4F4 A0A024R4F4_HUMAN;s	25	0	10500000	723030	8228500	411740	36654000	704920
sp P34932 HSP74_HUMAN;tr V9HW33	25	0	10096000	2625400	5326600	2512993	4076700	6741090
tr K7EIG1 K7EIG1_HUMAN;sp O75153	25	0	9287300	685700	4576200	1308700	18490090	1126600
sp Q9UPU5 UBP24_HUMAN	25	0	6638600	182400	4030600	433720	18350000	602200
sp Q7LBC6 KDM3B_HUMAN;sp Q7LBC	25	0	5956180	1054701	2931300	606990	44391930	4116782
sp O95239 KIF4A_HUMAN;tr Q59HG1	25	0	5698000	228260	4239970	1025200	34423000	989580
sp Q9H0A0 NAT10_HUMAN;sp Q9H0A	25	0	3689900	114400	2867500	185680	17843600	341920
tr Q53GA7 Q53GA7_HUMAN;sp Q9BQ	25	0	2303900	1244100	6387500	422190	10278000	1235700
tr V9HWD9 V9HWD9_HUMAN;tr Q53E	25	0	1247720	1836800	614900	680060	0	4857030
tr B3KUD7 B3KUD7_HUMAN	25	1	0	0	0	0	1271000	0
sp Q5T8P6 RBM26_HUMAN;sp Q5T8P6	25	0	0	0	344010	304360	2539500	1479700
sp P61978-3 HNRPK_HUMAN;tr B4DU	24	0	193875310	55376820	265959100	61099050	677014000	111298400
tr B5BUB1 B5BUB1_HUMAN;sp Q9Y26	24	0	162275870	57464510	248808930	47212020	304973800	52846300
tr B4DDF7 B4DDF7_HUMAN;tr A8K7B7	24	0	59449090	11997355	108627310	15776780	151149920	23674100
tr Q6NTA2 Q6NTA2_HUMAN;sp P1486	24	0	57483610	10801610	94082850	14688080	224096300	18008240
tr Q58EY4 Q58EY4_HUMAN;sp Q92922	24	0	30907590	1238700	10456300	695613	30468180	1675223
sp O00231 PSD11_HUMAN;sp O00231-	24	0	27674180	4701250	36216940	7587350	72897630	25652220



tr A0A024RCR6 A0A024RCR6_HUMAN;	24	0	27411110	4025700	12020000	7107000	26232000	5647000
sp Q96P70 IPO9_HUMAN	24	0	24438000	5610040	13915342	5553700	64762000	7291500
sp P53618 COPB_HUMAN;tr E9PP73 E	24	0	23541210	2718800	11390510	3172600	43796900	4262120
sp P27694 RFA1_HUMAN;tr I3L4R8 I3L	24	0	18796430	11422900	38142000	6264700	54194950	4958200
tr A0A024R6M3 A0A024R6M3_HUMAN	24	0	18319668	3785900	17026800	3329240	64693600	1844200
sp Q9BXJ9 NAA15_HUMAN;tr B2RBE5	24	0	17516950	1888900	8128450	1712500	25228590	3374555
sp P08243-2 ASN5_HUMAN;sp P08243	24	0	16449490	3381358	25488020	3110560	102490450	17152290
sp Q10567-2 AP1B1_HUMAN;sp Q1056	24	0	15826000	3742500	12901000	3320500	40720000	4690000
tr A8KA19 A8KA19_HUMAN;sp Q4359	24	0	14553000	2175150	7018900	2121900	56975480	5305090
sp Q96RL7-4 VP13A_HUMAN;sp Q96R	24	0	14423000	0	3503610	0	1880320	0
tr Q6IBN1 Q6IBN1_HUMAN;sp P61978	24	1	10434000	2242100	25235000	0	0	0
sp Q9UHB9 SRP68_HUMAN;sp Q9UHB	24	0	10151000	2116650	20073000	1845389	46548440	1029940
sp Q01780-2 EXOSX_HUMAN;sp Q017	24	0	8415253	997022	3448300	335560	47238510	6033780
sp Q95602 RPA1_HUMAN;tr B7ZKR9 E	24	0	8217100	409280	2808000	404760	25575000	8303060
sp Q13045-2 FUI_HUMAN;sp Q13045	24	0	6601100	607300	3068200	582570	54116000	1273900
sp Q43390 HNRPR_HUMAN;tr Q0VGD	24	0	6488142	2310730	12500100	2376420	63769770	6298300
sp Q94906-2 PRP6_HUMAN;sp Q94906	24	0	4413100	603070	5109900	0	35110230	1520700
sp Q06010-2 DIAP1_HUMAN;tr E9PHQ	24	0	3525600	871990	936230	871880	26000000	1235400
tr A0A024QZ63 A0A024QZ63_HUMAN;	24	0	2954000	548010	2929200	90051	19575000	689660
tr A0A024QZN4 A0A024QZN4_HUMAN	24	0	933700	499810	660150	858860	0	2526390
tr B2R959 B2R959_HUMAN;tr B4DPK8	24	1	0	0	0	0	1168500	0
tr B4DVK5 B4DVK5_HUMAN	24	0.000356	0	0	1075500	0	2055700	0
tr D3DS63 D3DS63_HUMAN;tr B3KT83	24	1	0	0	0	0	0	0
tr Q8IW6P Q8IW6P_HUMAN;tr B4DFH	24	0.003325	0	192200	0	134800	0	696260
tr A0A024RCM3 A0A024RCM3_HUMAN	23	0	143691440	63720590	285487080	56877130	793454700	102259800
sp Q9BVA1 TBB2B_HUMAN;tr A0A024	23	0	72112730	26658000	133817550	34232460	264509200	57605330
sp Q14157 UBP2L_HUMAN;sp Q14157	23	0	35122630	6232300	22382300	8189700	145858330	10513145
sp P07384 CAN1_HUMAN;tr A0A024R	23	0	22340560	12635319	46234770	8979460	40701530	8911030
sp P35606-2 COPB2_HUMAN;sp P3560	23	0	17216670	3041328	11058080	2016890	33933440	2403472
sp P45974-2 UBP5_HUMAN;sp P45974	23	0	16367720	5278700	12363000	4521698	68831760	8062921
sp P50570-2 DYN2_HUMAN;sp P50570	23	0	15995420	4819200	14748470	4862700	86663740	9294000
sp P17858 PFKAL_HUMAN;sp P17858-	23	0	13614970	4618070	29235540	3117440	56494470	8301575
sp Q8N1F7 NUP93_HUMAN;tr A8K897	23	0	11711780	1851700	21774000	3775800	58590600	3012910
sp P51532-5 SMCA4_HUMAN;sp P515	23	0	11166650	1259160	5009270	997980	35227690	1256800
sp Q00159-2 MYO1C_HUMAN;tr F5H6	23	0	10436338	842630	3691480	531990	10025000	185230
sp Q01813 PFKAP_HUMAN;sp Q01813	23	0	8140100	2911958	18594910	3221480	54127030	4139960
tr B2R8Z8 B2R8Z8_HUMAN;sp Q60506	23	0	7601260	2175800	11879661	742010	30652950	5102490
tr B7ZLC9 B7ZLC9_HUMAN;sp Q8TEQ6	23	0	7455850	605070	3799800	1254900	23631000	706268
tr X5D2F4 X5D2F4_HUMAN;sp Q7L576	23	0	7369300	586690	3552900	578010	25119000	1211100
sp Q00232 PSD12_HUMAN;sp Q00232	23	0	7215800	3703600	20664844	4092000	49511130	12079181
tr B7ZLZ7 B7ZLZ7_HUMAN;tr B3KMB1	23	0	6355600	3041580	3800500	531010	34889300	2020900
sp Q9UQ35 SRRM2_HUMAN;tr Q60382	23	0	4412500	0	2423800	547180	68284000	1526241
sp Q14690 RRP5_HUMAN;tr B4DH82 E	23	0	2664000	0	1670310	105280	15208000	0
sp P27816-6 MAP4_HUMAN;sp P27816	23	0	2269700	206459	989410	0	6444800	3657260
sp Q13885 TBB2A_HUMAN;tr B2R6L0	23	1	767260	0	0	0	3502700	0
tr Q8IUB0 Q8IUB0_HUMAN	23	0	711450	0	1876100	0	0	933400
sp P50570-3 DYN2_HUMAN;sp P50570	23	1	0	0	0	0	0	232030
sp Q14157-4 UBP2L_HUMAN;sp Q1415	23	1	0	0	0	312010	3878500	0
tr B4DY32 B4DY32_HUMAN	23	0.002678	0	438520	0	393470	7754100	623260
tr V9HVZ4 V9HVZ4_HUMAN;sp P0440	22	0	357187000	420744000	413976100	341832000	902478000	414182000
tr Q5JP53 Q5JP53_HUMAN;tr Q5SU16	22	0	165984010	57581350	322979330	68880210	667659000	104927600
sp Q43175 SERA_HUMAN;tr V9HW79	22	0	161911760	65297380	295976000	60087880	747130900	115705400
sp P23526 SAHH_HUMAN;sp P23526-2	22	0	52093660	21997460	136209090	34419920	237532890	41088100
sp P17812 PYRG1_HUMAN;tr B4E1E0	22	0	33230600	13093550	57879204	9627439	142521890	14758710
tr A0A024R718 A0A024R718_HUMAN;s	22	0	25367900	4533900	53198039	6653510	72689000	12286640
sp Q02790 FKBP4_HUMAN;tr B2R9U2	22	0	20015310	5926372	36074970	6029791	93222730	11812113
sp Q9BXP5-4 SRRT_HUMAN;sp Q9BXP	22	0	19325000	4824600	11227000	1609000	40739639	7146040
sp P09960 LKHA4_HUMAN;sp P09960-	22	0	15382000	8299643	42347928	6809549	77306240	8494586
sp Q9H9A6 LRC40_HUMAN	22	0	14844420	3418100	34405050	704870	57830400	1613000
tr B4DSH1 B4DSH1_HUMAN;tr B3KY60	22	0	12281000	1770600	5906540	665340	52358540	2249500
sp Q93009 UBP7_HUMAN;sp Q93009-	22	0	11469000	1563300	3337900	0	12282400	1870100
tr B7Z6F7 B7Z6F7_HUMAN;tr A0A024R	22	0	11378000	1054700	2623226	1755600	36202040	1686700
tr HOYM23 HOYM23_HUMAN;sp Q7517	22	0	10808700	1584055	7144200	1324600	40025910	713880
tr A8QI98 A8QI98_HUMAN;sp Q9Y2L1	22	0	10254000	1576700	5213000	1140700	50051200	4804800
sp Q8WUM0 NU133_HUMAN;tr B4E20	22	0	7140800	652520	4286700	219410	39848000	2327000
tr E9PD53 E9PD53_HUMAN;tr B3KXX5	22	0	6891600	1105800	1511900	940860	12564000	1464900
sp Q13620-1 CUL4B_HUMAN;tr K4DI9	22	0	5353800	2002310	4845600	791780	22119165	1621310
sp P35573 GDE_HUMAN;sp P35573-2	22	0	4119100	590480	2700600	658130	20647000	1953700
sp P54577 SYVC_HUMAN	22	0	2186510	0	1384400	782296	402850	6398900
tr I3L2B0 I3L2B0_HUMAN;tr I3L318 I3L	22	0.006554	830020	292770	655130	0	1694000	0
tr Q5U077 Q5U077_HUMAN;sp P07195	21	0	126152800	131659600	242003300	92546500	282464900	161244100
sp Q7Z3B4-3 NUP54_HUMAN;sp Q7Z3	21	0	53217590	20860000	145862257	16949530	110534930	9989200
sp P23396 RS3_HUMAN;tr Q53G83 Q5	21	0	49880700	39915100	103964700	26372300	488561900	60387600
tr A0A024RB85 A0A024RB85_HUMAN;s	21	0	47083590	20916400	83058000	15147300	198369800	38177400
tr E9KL35 E9KL35_HUMAN;sp P63244	21	0	21664550	18059900	35566760	11617490	48769010	19191870

sp P42566 EPS15_HUMAN;tr Q7Z5V0	21	0	15915000	1922200	8894000	4364400	11991000	5403750
sp P35998 PR57_HUMAN;tr Q75L23 Q	21	0	12279020	5981100	17730605	5409500	51363789	11001100
tr Q6IBR0 Q6IBR0_HUMAN;sp P04843	21	0	11907000	2681500	20249000	1077600	5659700	0
tr B7Z5S9 B7Z5S9_HUMAN;sp O94973	21	0	11830730	964650	8054400	1363300	25557000	1735200
sp Q7Z3K3-5 POGZ_HUMAN;sp Q7Z3K	21	0	11364000	1294500	4062347	754690	45122000	1920800
sp P78344 IF4G2_HUMAN;sp P78344-2	21	0	10327000	1402700	7944100	914740	29224000	1889400
tr A0A024R8A7 A0A024R8A7_HUMAN;	21	0	9895900	2018496	9334400	2041200	41586135	979720
tr Q6IAX5 Q6IAX5_HUMAN;sp P60228	21	0	9715230	5477519	18011182	3455498	25236000	3839963
sp Q8WWM7 ATX2L_HUMAN;sp Q8W	21	0	9528800	3381800	10180207	2484200	64544000	4948575
sp Q43707 ACTN4_HUMAN;tr Q96BG6	21	0	9277200	141600	862140	0	1182790	310360
sp P11388 TOP2A_HUMAN;sp P11388-	21	0	8210100	734450	3526600	923700	19023822	698450
tr Q6IBN0 Q6IBN0_HUMAN;sp Q4324-	21	0	7625900	2112300	12614810	2413387	40634000	4675285
sp Q14617 AP3D1_HUMAN;sp Q14617	21	0	6683600	0	4993750	0	37124890	376450
sp P17655 CAN2_HUMAN;tr Q59EF6 C	21	0	5279200	2786920	12236000	2469050	46532000	22290000
tr E9PKG1 E9PKG1_HUMAN;sp Q9987-	21	0	3065000	343620	7841280	953680	13544780	3300350
tr B3KQT9 B3KQT9_HUMAN;tr V9HVV-	21	0	1474770	2454100	5312500	2070898	820450	6769970
sp Q14966 ZN638_HUMAN;sp Q14966	21	0	1442100	431920	2977700	880480	25506000	1262800
sp P68366-2 TBA4A_HUMAN;sp P6836	21	0.001715	1381500	0	0	0	0	0
tr D3DTH7 D3DTH7_HUMAN;tr A1L180	21	0.006563	718060	237960	504740	0	1787900	0
tr B4DE59 B4DE59_HUMAN;tr C9JTX4	21	1	0	601620	0	745473	258880	724000
sp P63261 ACTG_HUMAN;tr B4E3A4 E	20	0	531882200	144523000	583207600	239965600	192862000	191495000
sp P07196 NFL_HUMAN;tr B3KQI5 B3	20	0	41790720	3243200	38647200	1672400	0	0
tr B2CIS9 B2CIS9_HUMAN;sp P31944	20	0	36765300	4746100	6159600	4229330	5990100	5824700
tr Q96IR1 Q96IR1_HUMAN;tr B2R491	20	0	30083620	13506700	55750700	9939160	389158250	21718030
sp Q92973-2 TNPO1_HUMAN;tr A0A0-	20	0	29591200	6550090	16090500	4305397	79241906	8414870
tr B4DVQ5 B4DVQ5_HUMAN;tr A1KYC	20	0	25132160	5209400	14636920	4484261	36316000	7535602
tr A0A024R1U0 A0A024R1U0_HUMAN;	20	0	23173280	8180205	30424710	9023048	24825000	3421671
tr Q9BR63 Q9BR63_HUMAN;sp Q9NSC	20	0	22886930	8552700	46816300	5731900	86923800	5468160
tr A0A087WTT1 A0A087WTT1_HUMAN	20	0	21898590	4736492	37146700	4790687	130467890	7274500
sp P61221 ABCE1_HUMAN;tr D6R9I9 E	20	0	19383870	10650330	36466580	5408225	88524590	5058188
tr B2RBI2 B2RBI2_HUMAN;sp Q96A49	20	0	15476250	16571232.8	75704520	22467090	61205870	53883000
sp P39023 RL3_HUMAN;tr Q8TBW1 Q	20	0	14370500	3805700	33793790	1698037.4	150584520	8459740
sp P55265-5 DSRAD_HUMAN;tr A0A0-	20	0	13739820	2559300	6830000	2205800	56215070	3987400
sp Q15233 NONO_HUMAN;tr A8K525	20	0	13501530	5717740	28656150	4112040	125870246	17180580
sp Q96KG9-3 NTKL_HUMAN;tr E9PS17	20	0	10929470	2447900	7312282	0	52426310	4908200
tr A8K2M0 A8K2M0_HUMAN;sp P436E	20	0	10859976	2853100	17291000	3513600	54595750	11372580
tr B2R8Y6 B2R8Y6_HUMAN;tr Q6M2M-	20	0	10109910	243140	2276500	345900	28935000	547940
tr A5PLK7 A5PLK7_HUMAN;tr A0A024-	20	0	10036870	2927278	14241870	1822167	22927290	7883790
tr B4DLG2 B4DLG2_HUMAN;sp O7515-	20	0	9478600	1993600	4130782	878130	40020320	3062173
tr B4DNN4 B4DNN4_HUMAN;sp P239-	20	0	9026730	5652440	17976290	5192305	13770100	3453180
sp Q6Y7W6-4 PERQ2_HUMAN;sp Q6Y-	20	0	8732800	2583700	8288400	3006400	26909000	4381612
tr A0A024R8W0 A0A024R8W0_HUMAN	20	0	7953800	3591960	11783460	2236400	30164620	5828890
sp Q9Y5L0-3 TNPO3_HUMAN;tr B2R6-	20	0	7225620	668250	5343500	719688	29616790	1280980
sp Q02952-3 AKA12_HUMAN;sp Q029-	20	0	7175500	1463863	3407700	2864600	14460000	3452500
tr A0A024RA61 A0A024RA61_HUMAN;	20	0	7011800	11545100	11357210	2797933	12894950	18259610
sp P15170-2 ERF3A_HUMAN;sp P1517	20	0	6619010	1955300	15867029	924780	36086780	2533654
tr Q53GK6 Q53GK6_HUMAN;tr Q53G9-	20	0	6470000	2312100	5284800	3190700	4347500	1391404
tr A0A024R6M2 A0A024R6M2_HUMAN	20	0	6210940	598083.9	1444200	261410	21685160	1162300
sp Q9BTC0 DIDO1_HUMAN;sp Q9BTC-	20	0	5960200	1155000	3025100	314070	18487000	324770
sp Q92576-2 PHF3_HUMAN;sp Q9257-	20	0	5822500	262120	1551600	1544600	13594000	927270
sp Q16513-2 PKN2_HUMAN;sp Q1651-	20	0	5616000	1221190	5074710	886740	41250560	2880050
tr A0A0A0MSQ0 A0A0A0MSQ0_HUMA	20	0	5345430	4194169	7526900	1985130	2035400	3166190
sp Q94832 MYO1D_HUMAN;tr J3QRN-	20	0	5226021	149830	3450800	73484	14511000	693150
sp O60271-4 JIP4_HUMAN;sp O60271-	20	0	3693200	646640	796470	644980	11925000	815160
sp P57740 NU107_HUMAN;sp P57740-	20	0	3285340	441040	3421400	220470	24733500	2133100
sp Q12789-3 TF3C1_HUMAN;tr Q6AHZ	20	0	2348800	0	911480	232080	13024000	0
tr B7ZME8 B7ZME8_HUMAN;sp Q641C	20	0	2324900	138900	393520	413610	45728220	1679200
tr Q6IAT1 Q6IAT1_HUMAN;sp P50395-	20	0	1883500	955200	1731000	576220	0	2402210
tr A0A087X054 A0A087X054_HUMAN;t	20	0	936080	0	590080	0	0	488790
sp P04350 TBB4A_HUMAN;tr B4DJ43 E	20	0	870940	225570	2337600	0	3982200	618200
tr Q59EC0 Q59EC0_HUMAN;tr B7Z8Y3	20	0.002729	634690	166340	650710	0	2229600	281690
sp Q14315-2 FLNC_HUMAN;sp Q1431-	20	0	355520	0	0	0	2277000	1686300
tr V9HW72 V9HW72_HUMAN;tr A8K6-	20	0	61899	169440	1011200	0	3107100	771350
sp Q9Y4E1-2 FA21C_HUMAN;sp Q9Y4E	20	0	0	0	0	0	2979900	0
sp Q15366-6 PCBP2_HUMAN;sp Q153-	19	0	77699400	25108360	167697700	28450750	383846600	56520280
tr F4ZW62 F4ZW62_HUMAN;sp Q1290	19	0	77154443	23050600	165898630	22203680	346922100	51582860
tr V9HWH2 V9HWH2_HUMAN;sp P12-	19	0	69768150	20835300	142690640	30433840	289822400	116346800
tr A0A024RD93 A0A024RD93_HUMAN;	19	0	54521824	54765120	88269260	26882670	80147410	42292870
tr A0A024R3X4 A0A024R3X4_HUMAN;s	19	0	38604520	13989854	71279133	11857713	13374000	2806521
tr A0A024RBY4 A0A024RBY4_HUMAN;s	19	0	33742424	10643880	64485110	9915210	153727500	19905310
tr R4GNH3 R4GNH3_HUMAN;tr A8K78	19	0	12400927	6840400	19686000	4520800	46215360	10546448
sp O00148 DX39A_HUMAN;tr B4DX78	19	0	12116610	2618900	22049000	2044556	44342060	2758400
tr A0A024R201 A0A024R201_HUMAN;s	19	0	11777891	0	31775880	5519100	56919400	13659518
tr Q53H17 Q53H17_HUMAN;tr Q53GN	19	0	11624420	314400	11813480	490746	36322340	4427290
sp P09651-3 ROA1_HUMAN;tr F8W6I7	19	0	11310310	8680550	21337828	3708487	130012440	17989790
sp Q9Y2W1 TR150_HUMAN;tr Q7Z5U1	19	0	10866450	1314500	5748660	1106600	31909000	3623670

sp P24928 RPB1_HUMAN;tr A0A087W	19	0	10279480	0	3964400	0	14127000	171750
tr D3DUP2 D3DUP2_HUMAN;tr F5GW1	19	0	10108000	1841700	5624300	3059300	13455000	615490
sp P16615-5 AT2A2_HUMAN;sp P1661	19	0	8816440	1486500	6399790	1001400	4083450	0
sp Q15020 SART3_HUMAN;sp Q15020	19	0	8286300	630240	2666600	469440	37431000	2004100
tr B4E0X8 B4E0X8_HUMAN;sp Q96AE4	19	0	7354130	11125670	9297981	7190680	15323000	11581590
sp Q15008 PSMD6_HUMAN;sp Q15008	19	0	7162700	636610	8562400	735860	20182000	2549814
sp Q14240 IF4A2_HUMAN;sp Q14240-	19	0	6356300	2419600	10997000	2445800	17877000	4693892
sp Q9H4A4 AMPB_HUMAN;tr A6NKB8	19	0	6112700	1873700	13580000	2165500	13914000	1386375
sp Q86UK7-2 ZN598_HUMAN;sp Q86U	19	0	6092900	1532550	5179200	1436050	23526190	3865040
tr E7EMW7 E7EMW7_HUMAN;sp O95C	19	0	5984100	236230	1638200	545510	16416050	394500
tr V9HWK0 V9HWK0_HUMAN;sp O76C	19	0	5716100	217040	6533600	366970	36530000	1032000
tr A0A090N8G0 A0A090N8G0_HUMAN	19	0	5607190	2865303	9917130	1416060	21378040	3307140
sp Q9HCE1 MOV10_HUMAN;tr Q5JR04	19	0	3298400	237737	2124000	0	19593540	689860
sp P49321 NASP_HUMAN;tr Q53H03 C	19	0	2233298	1285758	1013350	846205	6481800	6898830
tr A0A024R1K8 A0A024R1K8_HUMAN;s	19	0	1953825	758140	2658300	1140100	11019530	3126043
tr Q8N5A0 Q8N5A0_HUMAN;tr A0A08	19	0	1916600	713090	0	709270	7667000	1444900
sp P49750 YLP1_HUMAN;sp P49750-	19	0	1655100	171430	1636600	0	19657000	229940
tr Q6FIC5 Q6FIC5_HUMAN;sp Q9Y696	19	0	1347092	864952	4384740	2072000	63349600	4831190
tr D3DPU2 D3DPU2_HUMAN;tr B2RDY	19	0	361290	286300	811910	0	3828200	2656490
sp Q15366-3 PCBP2_HUMAN;sp Q1536	19	1	0	0	1091400	0	0	0
sp Q8IWZ3-6 ANKH1_HUMAN;sp Q8IW	19	0	0	0	0	0	3675300	0
sp P37802 TAGL2_HUMAN;tr X6RJ6 J	18	0	100919970	75145970	254754660	73336430	467942970	143316710
tr V9HWI5 V9HWI5_HUMAN;sp P2352	18	0	40598100	27599850	64147850	21071600	329901300	53755590
sp P52209-2 6PGD_HUMAN;sp P52209	18	0	40107888	13642156	80092730	12290000	126186060	22227380
sp Q06830 PRDX1_HUMAN;tr A0A0A0	18	0	27511600	22009200	26481700	20588600	62100700	62800800
tr A8K548 A8K548_HUMAN;sp Q8IZL8	18	0	26042580	1722464	6641830	7566600	75283760	3822930
sp Q43776 SYNC_HUMAN;tr B4DN60 I	18	0	23016470	4941210	36266000	6104925	91325840	8701212
sp Q9NZI8 IF2B1_HUMAN;sp Q9NZI8-	18	0	22652040	6277300	36099270	5532240	147103370	9409550
tr D0PNI1 D0PNI1_HUMAN;sp P63104	18	0	14802700	7666680	17193820	5393200	38625020	23459490
sp P62195-2 PRS8_HUMAN;sp P62195	18	0	13018865	5813120	24577460	7834714	32640780	12953569
sp P36578 RL4_HUMAN;tr Q59GY2 Q5	18	0	11904700	2397140	37946460	1380980	328615200	6125300
tr B7ZAX9 B7ZAX9_HUMAN;tr B4DZC0	18	0	7999500	1950900	8058059	667216	20729660	1933480
tr B4DSQ5 B4DSQ5_HUMAN;tr F5H365	18	0	7827293	2016960	11548000	1093850	31993000	3093180
tr I3LON3 I3LON3_HUMAN;tr B7Z5J7 B	18	0	7656900	3045826	17497000	2966260	51361430	3695800
sp Q9H2G2-2 SLK_HUMAN;sp Q9H2G2	18	0	7583600	1257300	3806300	1462400	36937000	2281500
tr Q59GB4 Q59GB4_HUMAN;sp Q1655	18	0	7186200	2094000	22980731	2475800	38126430	3415777
sp O00425 IF2B3_HUMAN;sp O00425-	18	0	7167000	3952600	13001735	2448200	72658801	4392960
tr Q53GV6 Q53GV6_HUMAN;tr B2R79-	18	0	7060950	4327630	13395140	4264710	61172610	13503318
tr A0A024RCR5 A0A024RCR5_HUMAN;	18	0	6040140	1125000	4123670	923410	44568000	3835810
tr Q53XC0 Q53XC0_HUMAN;sp P05198	18	0	5943100	3801026	13409470	2718957	114624640	9501920
sp P57737-2 CORO7_HUMAN;sp P5773	18	0	5769500	417020	3734800	597260	34468710	1566900
sp Q9Y2A7 NCKP1_HUMAN;sp Q9Y2A7	18	0	5570200	184140	3308920	704490	19012000	1472600
sp O15042-2 SR140_HUMAN;sp O1504	18	0	5570000	991292	5305060	1352300	41708000	1588400
sp P62424 RL7A_HUMAN;tr Q9BY74 C	18	0	5444580	2554500	13713840	979360	238211600	6534780
sp Q7Z460-2 CLAP1_HUMAN;sp Q7Z46	18	0	5006500	0	1018300	228270	13888000	0
sp Q8NC51-3 PAIRB_HUMAN;tr Q63HI	18	0	4300515.3	3254862	8268251.7	1639417	17628640	7016684
sp Q9NTI5-2 PDS5B_HUMAN;sp Q9NT	18	0	4234112	561320	1514800	268130	22141760	581820
tr A0A024R643 A0A024R179_HUMAN;s	18	0	4147427	606830	11489000	601587	30386000	970380
sp Q9Y6Y8 S23IP_HUMAN;tr B4DWG1	18	0	4125000	968750	3616300	666720	37177920	1694600
tr A0A090N7U0 A0A090N7U0_HUMAN	18	0	4086140	1254329	8606070	840594	17651955	1270523
sp Q9H2M9 RBGP_R_HUMAN;tr B3KNG	18	0	3872200	166720	2431100	0	20353743	1315300
tr Q2NKY5 Q2NKY5_HUMAN;sp Q9H4I	18	0	3094520	430179	5066290	0	13809820	323460
sp Q12996 CSTF3_HUMAN;tr B4DFQ5	18	0	2625500	1546687	10038000	2348320	20531330	5377400
tr A0A024RAV2 A0A024RAV2_HUMAN	18	0	2310870	432090	6037400	0	24742830	545210
sp Q9H1A4 APC1_HUMAN;tr H0Y564 I	18	0	2024800	245707	881620	269870	11392250	530710
sp Q12768 STRUM_HUMAN;tr Q53EL1	18	0	1615300	213900	2384100	0	24549000	368590
tr B1ANR0 B1ANR0_HUMAN;sp Q1331	18	0	1109800	469430	3832400	0	24039000	479754
sp Q9BUF5 TBB6_HUMAN;tr B4DP54 I	18	0	760520	0	1373000	0	5856100	0
sp O14776-2 TCRG1_HUMAN;sp O1477	18	0	641020	0	0	0	6587600	470940
tr B4EQQ4 B4EQQ4_HUMAN;tr B4DZJ7	18	0	409190	0	0	0	0	55850
tr B5BUB5 B5BUB5_HUMAN;sp P05455	18	0	207480	1159720	385450	157920	344960	3360130
tr A0A024R643 A0A024R643_HUMAN	18	1	0	0	413570	0	699790	0
tr Q9UQM3 Q9UQM3_HUMAN	18	0	0	0	0	0	699650	0
sp P26599 PTBP1_HUMAN;sp P26599-	17	0	93496200	23624640	165240510	24153859	255150110	61736350
tr Q7Z726 Q7Z726_HUMAN;tr Q6NVVW	17	0	61514530	19856870	114698322	18971050	69473380	12272820
tr V9HW24 V9HW24_HUMAN;sp P623	17	0	23247000	2213800	42282000	7795100	57700960	11917535
tr A0A024R5Q7 A0A024R5Q7_HUMAN;	17	0	21639000	6457000	41734000	6601300	64538910	7409128
tr A0A024R0R4 A0A024R0R4_HUMAN;s	17	0	15961870	493740	22563000	2101200	58728880	11478791
tr A0A024R4G1 A0A024R4G1_HUMAN;	17	0	10621190	3358500	22069000	2181000	50058000	1656667
sp Q9Y3F4 STRAP_HUMAN;sp Q9Y3F4	17	0	8789336	402610	18232000	2693660	34086100	11634080
sp Q8WX92 NELFB_HUMAN	17	0	8596982	2341500	11282000	1723100	37902590	977524
sp Q13435 SF3B2_HUMAN;tr E9PPJ0 E	17	0	7278300	903340	2639305	362660	25827000	2648000
tr G5EA31 G5EA31_HUMAN;tr A0A024	17	0	6836869	1444200	4431000	1121400	37407080	3236500
tr X5D2E5 X5D2E5_HUMAN;sp Q86WJ	17	0	5932400	930190	2956400	1012620	45411060	2471300
tr A0A024R8V0 A0A024R8V0_HUMAN;	17	0	3844900	6279700	10099850	10267200	49397300	19231600
tr Q3BDU5 Q3BDU5_HUMAN;sp P0254	17	0	3676445	0	0	745050	551920	1485601
tr A0A024RC57 A0A024RC57_HUMAN;s	17	0	3143000	1407200	7316100	1257800	30868200	1005200
tr E7ER18 E7ER18_HUMAN;tr E7EW49 I	17	0	3041900	344520	1766300	193490	17862000	350750



tr A8K3W4 A8K3W4_HUMAN;tr A0A02	17	0	2931960	221970	1265843	156330	24964300	875450
tr D9YZV0 D9YZV0_HUMAN;tr B3KN85	17	0	2473210	24798000	15789500	23284600	29227500	27873600
tr Q53EQ3 Q53EQ3_HUMAN;sp Q9BSJ	17	0	2422580	356480	3150104	509010	15319000	544950
sp Q6PGP7 TTC37_HUMAN;tr D6RCE2	17	0	2421890	100310	1409400	238930	13136070	210490
sp P62136 PP1A_HUMAN;sp P62136-2	17	0	2274810	4765010	18128000	1816100	95684000	13949390
sp Q96JM3 CHAP1_HUMAN;tr S4R3K0	17	0	2178600	816040	1140800	491710	20743000	1889000
sp Q5SRE5-2 NU188_HUMAN;sp Q5SR	17	0	2173700	0	2877220	157700	9366900	120290
tr A0A024R542 A0A024R542_HUMAN;s	17	0	1783900	120060	384730	154840	10966000	266810
tr HOY4W2 HOY4W2_HUMAN;tr F2Z2U	17	0	1628200	0	482023	0	12352000	0
tr V9HWC0 V9HWC0_HUMAN;sp P26C	17	0	520370	342720	0	393180	0	2343850
tr V9HWE0 V9HWE0_HUMAN;sp P087	17	0	0	1801780	0	592140	791300	6239540
sp P31943 HNRH1_HUMAN;tr G8JLB6	16	0	72014980	21712793	122936960	25528850	248630800	46411510
tr Q53SS8 Q53SS8_HUMAN;sp Q15365	16	0	56519850	8100510	102627070	16513690	208985580	41823520
sp Q9BZZ5-2 API5_HUMAN;sp Q9BZZ5	16	0	51188860	8170320	62802249	9883830	216828650	24511190
sp Q9UMS4 PRP19_HUMAN;tr F5GY56	16	0	17669882	2849830	38465274	2436700	84298520	10069232
sp Q9Y3I0 RTC8_HUMAN;tr B4DNA0 E	16	0	17068998	3372051	20627380	2911000	80388860	5394070
tr A0A087WUA5 A0A087WUA5_HUMA	16	0	14327087	6457030	34280860	5092100	3037300	2181334
tr B5BU72 B5BU72_HUMAN;tr A0A024	16	0	14320030	4213300	32549000	3287807	47532600	1121092
sp P06748-2 NPM_HUMAN;sp P06748	16	0	11695710	11123700	25011420	3388890	203632680	28953570
tr Q0VGA5 Q0VGA5_HUMAN;tr Q53H	16	0	11506770	2321900	21783000	5319890	62906000	10641620
tr A0A024R426 A0A024R426_HUMAN;s	16	0	10764470	6625837	27831330	1844900	129131580	10956320
sp Q6UWP8 SBSN_HUMAN;sp Q6UWF	16	0	9950500	6096200	7385700	8960900	6714800	7583100
sp P51659 DHB4_HUMAN;tr E7ER27 E	16	0	9654190	5315370	20603000	4471700	20890990	3400130
sp Q96T58 MINT_HUMAN;tr HOY5U7 H	16	0	9403690	1184840	2261800	584670	5967400	0
tr C9J2Y9 C9J2Y9_HUMAN;tr B4DHJ3 E	16	0	8192600	558520	2909100	361580	16511000	1009900
sp O60701 UGDH_HUMAN;sp O60701-	16	0	7466040	2266800	14282000	2363220	40127390	2799165
tr V9HW98 V9HW98_HUMAN;sp P622	16	0	7009650	6165570	26639000	3071250	29188800	17974020
sp Q43290 SNUT1_HUMAN;tr B4DDH9	16	0	6722800	1496866	5518220	904660	36551720	2736738
tr B2RNR6 B2RNR6_HUMAN;sp Q96KF	16	0	6528700	1593300	5882500	430230	39371680	3055700
sp Q06210-2 GFPT1_HUMAN;sp Q0621	16	0	5908200	2669400	4571600	1691628	31256000	2671992
sp P14735 IDE_HUMAN;tr Q59GA5 Q5	16	0	5885472	357610	522620	442410	15262140	1126400
sp Q05639 EF1A2_HUMAN;tr Q59GP5	16	0	5451022	922950	6854800	901460	8835900	2780100
tr A8K9U6 A8K9U6_HUMAN;sp Q7Z2W	16	0	5438550	1611530	4254520	352718	17861900	904504
sp Q15046 SYK_HUMAN;sp Q15046-2	16	0	5419900	2326193	7038900	1826064	22675450	3211210
sp Q96T88 UHRF1_HUMAN;tr A0A087	16	0	5403770	1532900	4556990	545625	17388540	1879770
sp P26358 DNMT1_HUMAN;tr A0A024	16	0	4513500	2721290	2693300	1839470	22550020	2578870
sp Q9H2U1-2 DHX36_HUMAN;sp Q9H2	16	0	4295900	266110	1723900	370290	16279545	0
tr E5KLB5 E5KLB5_HUMAN;sp P49916	16	0	4226800	462090	2107400	128570	16853000	715948
sp Q9H3U1-2 UN45A_HUMAN;sp Q9H	16	0	4042340	855740	3836300	620780	11423900	927760
tr Q2M1V9 Q2M1V9_HUMAN;tr A0A0	16	0	4006730	0	1239900	0	14778190	62408
tr B2RDD7 B2RDD7_HUMAN;sp Q1474	16	0	3680800	2321600	6638000	2618837	5897000	2222170
tr Q53F17 Q53F17_HUMAN;sp Q13642-	16	0	3413390	3389000	8297600	430960	80531600	3072080
sp P18858 DNL1_HUMAN;tr F5GZ28 F	16	0	3369300	739120	2525200	521570	24259940	466990
tr A0A024R0Y5 A0A024R0Y5_HUMAN;s	16	0	3340700	1178900	9077400	1125400	17448000	2797600
sp Q27J81-2 INF2_HUMAN;sp Q27J81	16	0	1969100	208520	2614200	272170	24105000	379180
tr A0A024R8S5 A0A024R8S5_HUMAN;s	16	0	1644837	0	395740	220540	0	0
sp P35249 RFC4_HUMAN;sp P35249-2	16	0	1612300	1171630	7797300	1009600	38471200	1367542
tr Q53ZP9 Q53ZP9_HUMAN;sp O95757	16	0	1544300	820440	1046500	429350	0	1144600
sp Q13619 CUL4A_HUMAN;sp Q13619	16	0	1523800	485940	4411000	582720	6854600	0
tr V9HW04 V9HW04_HUMAN;sp P621	16	0	1127100	1313100	3781500	922750	32072000	2186600
sp A6NKT7 RGPD3_HUMAN;tr J3KNE0	16	0.002691	831370	0	1143500	0	1710800	161920
tr A0A024R222 A0A024R222_HUMAN;s	16	0	541320	435931	0	534825	4913200	4602720
tr HOY4R1 HOY4R1_HUMAN;sp P12268	16	0	272250	405145	694710	221072	0	2348210
sp P26358-2 DNMT1_HUMAN;tr I6L9H	16	1	0	0	0	0	435740	0
sp Q9BZZ5 API5_HUMAN;tr G3V1C3 C	16	0.00363	0	0	0	0	513320	0
tr B4DNH2 B4DNH2_HUMAN;tr J3KTN	16	1	0	0	388480	0	0	0
tr Q6NUR7 Q6NUR7_HUMAN;tr E7EQF	16	0	0	0	0	0	0	840460
sp Q9NZT1 CALL5_HUMAN;tr Q53H37	15	0	118812000	53358000	35790000	26915700	50570000	31410000
tr E7D7VW5 E7D7VW5_HUMAN;sp Q014	15	0	114243600	19735000	31803400	20002000	82614200	32940100
sp Q08188 TGM3_HUMAN	15	0	27387810	6286900	5433370	10216250	3480810	9153930
sp Q96EA4 SPDLY_HUMAN;sp Q96EA4	15	0	25628357	0	15846150	0	9765700	0
sp Q8WVV4 POF1B_HUMAN;sp Q8WV	15	0	21586190	694760	252050	1425830	104910	892222
sp P16152 CBR1_HUMAN;sp P16152-2	15	0	17471110	16397720	36161130	8524556	258198610	23926470
tr B4DRA0 B4DRA0_HUMAN;sp Q1449	15	0	17286550	9167570	47700812	3998619	126188940	6545080
tr V9HW89 V9HW89_HUMAN;sp Q007	15	0	13736310	1080510	4430231	3441679	55692000	8822280
tr A0A024R4U3 A0A024R4U3_HUMAN;	15	0	13260000	4197761	15745160	4552766	52694000	7439970
sp P13489 RINI_HUMAN;tr A0A024RC	15	0	13217000	4321600	24105835	4260540	49937020	6979460
sp P15880 RS2_HUMAN;tr Q8N5L9 Q8	15	0	13176140	2791224	27589810	1705780	372108040	10704270
tr A0A024RDV7 A0A024RDV7_HUMAN	15	0	12786476	5070530	33390500	3950700	78333259	8505910
sp P49419-2 AL7A1_HUMAN;sp P4941	15	0	12211000	4166763	18193580	2460340	41475000	3979960
sp Q14677-3 EPN4_HUMAN;sp Q1467	15	0	12126250	7557031	20748273	6277500	35134920	8078500
sp Q57749 KPRP_HUMAN	15	0	12071740	8000700	4196450	7186700	12056080	17611380
tr Q5SRT3 Q5SRT3_HUMAN;tr Q53FB0	15	0	11371500	18784468	27912993	10087550	212339140	39679160
tr E9PIR7 E9PIR7_HUMAN;tr B2R5P6 E	15	0	9806570	1917900	16835340	1338000	45458460	3275399
tr V9HWK1 V9HWK1_HUMAN;sp P601	15	0	9053820	7868670	5415500	5666900	9391100	20146000
tr Q9BV28 Q9BV28_HUMAN;tr B2RBD	15	0.000369	8707000	0	0	0	0	0

sp P50995-2 ANX11_HUMAN;tr Q5T0C	15	0	8307823	5818450	16979000	7209000	21823900	11400960
sp Q9UBT2 SAE2_HUMAN;tr B2RDF5 E	15	0	7916290	3846483	16020380	1880907	17460560	4600100
sp O14497 ARI1A_HUMAN;sp O14497-	15	0	6936000	618350	2983000	542070	9343720	157530
tr A0A0A0MTB8 A0A0A0MTB8_HUMAN	15	0	6547500	1741860	5804900	1741780	5441300	0
sp P23258 TBG1_HUMAN;sp Q9NRH3	15	0	6136400	925750	14024472	906270	32351000	3448098
sp P18124 RL7_HUMAN;tr A0A024R81-	15	0	6103140	3190890	19266550	1608840	301182460	3846120
sp Q99615 DNJC7_HUMAN;tr Q59EH7	15	0	5451900	2099700	10789660	3639677	14646000	1965784
tr Q6IBR2 Q6IBR2_HUMAN;sp Q9Y285	15	0	5282260	3741886	14867812	4051600	28282100	5785400
tr H7BXH2 H7BXH2_HUMAN;tr E9PKF6	15	0	5276000	102780	2627000	307290	21922000	1031800
sp P39880-2 CUX1_HUMAN;sp P39880	15	0	4962430	539870	2802000	147150	3179120	200500
sp P04040 CATA_HUMAN;tr B4DWK8	15	0	4884540	3933830	3594550	5853010	2158310	3959830
tr B4DYP7 B4DYP7_HUMAN;sp Q96CW	15	0	4813100	727370	3629300	231390	23380270	605930
tr Q9HC39 Q9HC39_HUMAN;tr Q9HBB	15	0	4588600	357910	2321200	495960	13450000	818490
sp P36871 PGM1_HUMAN;tr B4DFP1 I	15	0	4563100	1446200	15598454	726801	31200370	3831470
sp Q96ST3 SIN3A_HUMAN;tr B3KQE3	15	0	4258000	344410	1567300	196150	9877600	681980
tr J3KNL6 J3KNL6_HUMAN;sp O15027	15	0	4210375	632660	4706610	831710	12912890	677970
tr A0A024R2G2 A0A024R2G2_HUMAN;	15	0	4065900	100070	1028000	0	8225800	142160
sp Q6PKG0 LARP1_HUMAN;sp Q6PKG	15	0	4005800	270550	974338	487080	12670640	2022660
sp Q9NZB2 F120A_HUMAN;sp Q9NZB	15	0	3983400	903890	5107490	61333	20090620	1640300
sp P09884 DPOLA_HUMAN;tr A6NMQ	15	0	3744200	186850	1279100	399960	13542000	747740
tr Q6IB98 Q6IB98_HUMAN;sp O15372	15	0	3594600	108680	15848700	785599	14011900	1395214
sp Q9Y295 DRG1_HUMAN;tr Q9UFA5	15	0	3411600	479640	10207610	256440	21372000	2734933
sp Q9UNE7 CHIP_HUMAN;sp Q9UNE7	15	0	3375270	3306145	7598250	1807586	41727000	3410400
tr Q6ZMY0 Q6ZMY0_HUMAN	15	0	2923532	0	470140	0	15090000	0
sp Q9UK61-3 F208A_HUMAN;sp Q9UK	15	0	2740500	138470	462980	343720	12545780	137540
sp Q8WUA4 TF3C2_HUMAN;tr Q53QN	15	0	2480800	74060	668620	0	16785320	756990
tr A0A024R475 A0A024R475_HUMAN;s	15	0	2328580	363440	5794200	355680	12776800	1563570
sp Q96L91-3 EP400_HUMAN;sp Q96L9	15	0	2197400	0	1229200	44571	7511700	0
sp Q9BY44-3 EIF2A_HUMAN;sp Q9BY4	15	0	2160100	255700	4889600	693560	19679000	750110
sp Q10570 CPSF1_HUMAN;tr D3DWL9	15	0	2098600	350170	1399600	463560	12005240	573510
tr Q6IAP9 Q6IAP9_HUMAN;tr Q5T1M7	15	0	1459600	3357659	14677000	4529700	35035961	6173900
sp Q9HC35 EMAL4_HUMAN;tr B5MBZ	15	0	1424800	165470	632860	0	12252360	626570
tr A0A024R2Z6 A0A024R2Z6_HUMAN;s	15	0	1213300	198760	3881900	0	23881000	55333
sp Q6P1N0 C2D1A_HUMAN;sp Q6P1N	15	0	1051000	793517	1569393	3351800	6820400	1022800
sp Q8TAQ2-2 SMRC2_HUMAN;sp Q8T	15	0	968120	165420	544060	246790	12923406.6	233860
tr B9A6J2 B9A6J2_HUMAN;sp Q15042	15	0	963900	111930	1122500	294690	13311000	201400
tr Q9UPN1 Q9UPN1_HUMAN;tr F8VYE	15	0	769310	840900	3075310	767080	18524000	312581
tr V9HWH6 V9HWH6_HUMAN;sp P004	15	0	541100	64278	0	229700	563180	1026174
sp P63241 IF5A1_HUMAN;tr I3L397 I3	14	0	72448590	55798109	45385610	27433950	340028430	38916080
sp P07737 PROF1_HUMAN;tr K7E144 I	14	0	66733470	36016190	139953572	29617750	718661140	77883290
sp P62826 RAN_HUMAN;tr B5MDF5 B	14	0	51150400	47619960	83915500	35318610	39487250	17626290
sp P27348 I433T_HUMAN;tr B4DMT8	14	0	18921000	2568484	15606000	649520	20238670	6911220
tr Q53XL8 Q53XL8_HUMAN;tr Q53HB3	14	0	18358580	2632900	37337000	6655788	68150790	16865340
tr B4DWI8 B4DWI8_HUMAN;sp Q8WX	14	0	16226000	4873200	27342000	4052076	107553220	5850364
tr C9J9K3 C9J9K3_HUMAN;tr A0A024R	14	0	15861630	1028790	7217860	1604400	21777760	15351620
sp P62269 RS18_HUMAN;tr Q5GGW2	14	0	14371680	9212829	35563040	7281876	229374540	24140910
sp Q7L2H7 EIF3M_HUMAN;sp Q7L2H7	14	0	13422000	791230	20549000	2041400	34813380	6229050
tr Q4VB24 Q4VB24_HUMAN;tr A3ROT8	14	0	12981300	8628300	20966800	3965500	245119500	13411400
tr Q6NXR8 Q6NXR8_HUMAN;sp P6124	14	0	10496510	8287449	25724960	5903870	223190000	10606310
sp Q86TB9 PATL1_HUMAN;sp Q86TB9	14	0	10434300	990720	5780760	286880	12322120	0
sp Q15645 PCH2_HUMAN;tr HOYAL2 H	14	0	8430570	3391900	18217000	3853710	34593500	5125180
sp P49756 RBM25_HUMAN;sp P49756	14	0	8392100	915090	5802800	294480	40875000	2550500
sp Q9UBB4 ATX10_HUMAN;sp Q9UBB	14	0	8107530	2008500	9614100	2802700	30288000	4464500
sp P61011 SRP54_HUMAN;tr G3V480	14	0	7973200	1982300	14606000	1823500	25504000	4454800
tr B2R858 B2R858_HUMAN;sp P26196	14	0	7237080	3115400	16102000	2825400	30263730	3632700
sp P04844-2 RPN2_HUMAN;tr B2RE46	14	0	7043980	1094800	11515000	279140	3901100	0
sp Q92878-3 RAD50_HUMAN;sp Q928	14	0	5067500	2730210	1922300	628760	21318600	1469300
tr A0A024R395 A0A024R395_HUMAN;s	14	0	4979703	618820	8104756	413548	19511480	1527590
tr Q53FW2 Q53FW2_HUMAN;sp P608	14	0	4537816	8229090	20572445	3586624	105780000	13228610
sp Q86Y56 DAAF5_HUMAN;sp Q86Y56	14	0	4387980	1221100	6678000	2130900	12710700	1057700
sp O15397 IPO8_HUMAN;sp O15397-2	14	0	4246400	456110	2279310	0	17987000	716550
sp Q9BTW9 TBCD_HUMAN;tr J3KR97	14	0	3962700	153620	2555500	286560	12421000	698410
sp P46087 NOP2_HUMAN;sp P46087-4	14	0	3883100	407560	1327500	49122	20064000	700140
sp P42858 HD_HUMAN;tr D3DVR8 D3	14	0	3741900	0	289410	0	6382100	86558
sp Q9BQ52-4 RNZ2_HUMAN;tr B4DT1	14	0	3631900	352996	1008900	0	19587260	861810
sp P19623 SPEE_HUMAN;tr K7ESLO K7	14	0	3539300	4110700	8452800	2601090	60387000	6482970
tr A1L3A9 A1L3A9_HUMAN;sp Q66K14	14	0	3456700	261121	2126400	475010	12401000	443670
tr A0MNN4 A0MNN4_HUMAN;sp Q2T	14	0	3169140	565350	4283300	175300	24437060	928885
tr Q7RTQ9 Q7RTQ9_HUMAN;tr Q7RTC	14	0	3004700	195250	3114400	124010	14616000	755498
sp Q9UHX1-4 PUF60_HUMAN;sp Q9U	14	0	2919800	2509600	28645000	1318900	42483000	6086612
tr B3KY11 B3KY11_HUMAN;tr A0A024F	14	0	2914800	507550	3966100	0	31957400	505300
tr A0A024R6D1 A0A024R6D1_HUMAN;	14	0	2837500	677021	1111400	577640	25675680	2125800
tr A0A024R2Q4 A0A024R2Q4_HUMAN;	14	0	2823770	2376720	7602230	1095970	100303350	3597760
sp Q95782-2 AP2A1_HUMAN;sp O957	14	0	2822695	558350	2438151	19985	3621200	0
sp Q9Y371 SHLB1_HUMAN;sp Q9Y371-	14	0	2448400	3516100	31267000	8960900	67216910	46414310

sp P62495-2 ERF1_HUMAN;tr B7Z7P8	14	0	2382800	1089000	3574800	144112	3878400	1327300
sp Q43795-2 MYO1B_HUMAN;tr E9PD	14	0	2345500	247480	2170300	0	8950070	0
tr Q6FHU2 Q6FHU2_HUMAN;sp P1866	14	0	2176188	1282374	147610	660410	2456200	3798970
sp P19367-4 HXK1_HUMAN;tr B4DG62	14	0	2082100	718740	1834700	0	10394000	131940
tr B2RTX8 B2RTX8_HUMAN;sp Q7Z5K2	14	0	1949600	0	1616343	0	12995100	268430
tr Q9NPK3 Q9NPK3_HUMAN;tr O7604	14	0	1439800	0	543770	460330	10375000	0
tr B3KUN1 B3KUN1_HUMAN;sp P6777	14	0	1268580	2796200	5614279	1368400	64312000	4078400
sp Q9Y4B6-2 VPRBP_HUMAN;sp Q9Y4	14	0	1080000	121860	715690	110950	13142000	235650
sp Q15785 TOM34_HUMAN;tr B4DXU2	14	0	1050400	669150	6856300	856990	30081000	714700
tr A8K2R3 A8K2R3_HUMAN;sp O60341	14	0	941220	111020	0	0	6277800	277950
tr Q5U0F4 Q5U0F4_HUMAN;tr Q53HU	14	0	869380	1206800	4025600	478630	23264090	1973280
tr V9HW21 V9HW21_HUMAN;sp P009	14	0	806270	5499340	1623000	2826080	3160090	18327460
sp P40937-2 RFC5_HUMAN;tr Q6LES9	14	0	780460	0	1257360	217471	19960400	1734800
sp Q9P265 DIP2B_HUMAN;tr Q96IB4	14	0	689030	195210	2463900	139310	13376000	333330
tr Q5SQH4 Q5SQH4_HUMAN;sp O6022	14	0	637670	645340	2394600	829350	16895000	2881800
sp Q7Z406 MYH14_HUMAN;sp Q7Z406	14	0	216736	1173800	0	892980	28715000	1941300
sp Q14232 EI2BA_HUMAN;sp Q14232-	14	0	144820	311257	1359800	81643	17175000	1756758
tr A8KAK1 A8KAK1_HUMAN;sp Q9NYU	14	0	0	0	0	0	0	460500
sp P37837 TALDO_HUMAN;tr F2Z393	14	0	0	836760	369800	80307	579200	1724570
tr V9HW63 V9HW63_HUMAN;sp Q131	14	0	0	229510	121120	62111	1401600	1355100
tr Q9UM02 Q9UM02_HUMAN;tr B2RAI	14	0	0	0	0	4057000	0	8496500
tr MOR0F0 MOR0F0_HUMAN;tr A0A024	13	0	62008630	15703662	74616120	13447110	304420660	30063370
tr A0A024RDF4 A0A024RDF4_HUMAN;	13	0	45384300	14524730	80339320	16286150	173063200	33496290
sp P25311 ZA2G_HUMAN;tr C9IEV0 C	13	0	41092600	19371200	20236800	23095300	22253000	19424900
tr V9HWF5 V9HWF5_HUMAN;tr A8K44	13	0	38869330	22981870	61449060	11864430	211078080	61731930
sp P27824 CALX_HUMAN;sp P27824-2	13	0	28686420	7555800	40289910	8988320	17519000	1951994
tr Q53FC7 Q53FC7_HUMAN;tr B3KSM6	13	0	24510660	21047720	53781700	20700500	22015100	9962290
tr A0A024R7T3 A0A024R7T3_HUMAN;s	13	0	23327000	6814800	22993109	3471500	37729970	7144190
sp P55036 PSMD4_HUMAN;tr Q5VWC	13	0	19560188	4029200	28506000	5103500	59372870	8619950
tr Q5VVD0 Q5VVD0_HUMAN;sp P6292	13	0	16079750	11671400	28544960	9947540	158972010	25190600
tr Q6FHF5 Q6FHF5_HUMAN;sp P12004	13	0	12282740	11169130	33052200	8148860	229922200	21886349
sp P62906 RL10A_HUMAN;tr Q1UQ76	13	0	11386890	8999100	24832122	5664350	284420654	17681590
tr B0YIW5 B0YIW5_HUMAN;sp P48444	13	0	11318750	2023200	22837000	2790749	50892680	4616276
sp O15371-2 EIF3D_HUMAN;sp O1537	13	0	11205320	2333190	19383750	1777740	38215290	2426068
sp P23381-2 SYWC_HUMAN;tr A0A024	13	0	10571450	5790600	19421000	2568500	32384000	8823906
sp P11387 TOP1_HUMAN;tr B9EG90 B	13	0	9216700	1292723	1066500	0	38688260	1380600
tr A0A024RAI1 A0A024RAI1_HUMAN;s	13	0	8636400	4325000	14111000	3179000	36944280	3099460
tr V9HWE7 V9HWE7_HUMAN;sp P515	13	0	8621400	1216000	19345000	3514600	37653000	5291090
tr G3V4W0 G3V4W0_HUMAN;tr B4DYU	13	0	8437530	2062570	10025958	2542420	96472320	14995300
sp Q13363-2 CTBP1_HUMAN;tr X5D8Y	13	0	8184540	1787670	17449640	2380759	36462400	4373025
sp P51114 FXR1_HUMAN;sp P51114-2	13	0	7183680	3228840	10760000	346580	27501700	1093750
sp Q12904 AIMP1_HUMAN;sp Q12904	13	0	6225660	0	848510	1448600	61722663	3507700
sp P16403 H12_HUMAN;sp P16402 H1	13	0	5769000	635980	1159563	1320502	10379460	8715590
tr B3KMV5 B3KMV5_HUMAN;tr A0A024	13	0	3644300	521300	2392800	565720	2160190	55047
sp P20700 LMNB1_HUMAN;tr Q6DC98	13	0	3559000	1982900	4161800	1496000	0	0
sp Q99567 NUP88_HUMAN;tr B7Z5I6	13	0	3360500	809930	11012600	876310	31402000	1608695
tr Q86X55-1 CARM1_HUMAN;sp Q86X	13	0	3319230	1988693	8816100	911930	17183890	3278100
tr A0A024R4M0 A0A024R4M0_HUMAN	13	0	3133360	523776	7220250	516820	123022640	3904350
sp O75400-2 PR40A_HUMAN;sp O7540	13	0	3109900	357530	3528704	59582	34593690	2106000
sp Q15717 ELAV1_HUMAN;sp Q15717-	13	0	3066520	2537546	7410750	1559742	44241630	1747689
sp O75475 PSIP1_HUMAN;sp O75475-	13	0	2887600	1988867	4938552	2523600	17412800	2437735
sp P17480-2 UBF1_HUMAN;tr E9PKP7	13	0	2783910	402300	0	145280	24761100	1535600
sp O75717 WDHD1_HUMAN;tr A8KAE	13	0	2718700	133680	1282104	0	10826000	855120
sp Q9P2R3 ANFY1_HUMAN;sp Q9P2R3	13	0	2710900	369680	2552800	204280	13418000	522880
tr A0A024R0A8 A0A024R0A8_HUMAN;	13	0	2487390	507720	3508800	483390	16125800	1398500
sp Q9Y6M1-1 IF2B2_HUMAN;sp Q9Y6M	13	0	2320100	717170	1353800	752462	18928000	3309681
sp Q8NE71 ABCF1_HUMAN;tr Q2L6I2	13	0	2230950	206550	458580	240970	3548500	565850
tr Q53FV3 Q53FV3_HUMAN;tr B3KM4	13	0	2085100	894030	6389700	1607865	21531000	4779420
tr A0A024R6Q1 A0A024R6Q1_HUMAN;	13	0	1893884	511980	4025800	417900	22602658	2473700
tr E9PCY5 E9PCY5_HUMAN;tr B4DKD0	13	0	1868500	0	1458400	571120	4217800	116830
sp Q96F86 EDC3_HUMAN;tr H3BPPW9	13	0	1768000	317280	4549000	799260	12931000	265780
sp Q6KC79-2 NIPBL_HUMAN;tr Q6IEH	13	0	1749900	0	893110	0	9727700	0
sp Q86U86-8 PB1_HUMAN;sp Q86U86	13	0	1729500	3950130	764540	4603140	7481844	5869970
tr Q5TZP7 Q5TZP7_HUMAN;sp P27695	13	0	1616380	2994050	8184200	1673636	18544090	6586790
tr A8K5T7 A8K5T7_HUMAN;sp Q9Y2Z0	13	0	1471420	568626	7982300	1767653	23628000	4738815
sp Q8N6T3 ARFG1_HUMAN;tr Q53F62	13	0	1347100	3083123	17726600	5130346	55239720	22521913
sp P06493 CDK1_HUMAN;tr A0A024Q2	13	0	1163180	2326230	3925310	1693980	11757900	2613150
tr E9PHA2 E9PHA2_HUMAN;tr A8K4T8	13	0	1020300	46819	1936360	304740	19659110	2262000
tr Q6DEN2 Q6DEN2_HUMAN;sp Q1414	13	0	1017400	312990	2747300	698460	15046000	1625900
tr A0A024R7A8 A0A024R7A8_HUMAN;	13	0	878090	2147500	3164130	1001513	11747900	5631120
tr A0PJ92 A0PJ92_HUMAN;tr A8K9K6	13	0	650450	0	736630	0	14328000	0
tr A0A024R0R9 A0A024R0R9_HUMAN;s	13	0	610950	1064500	2297300	0	20063000	1566800
tr A0A024RC67 A0A024RC67_HUMAN;s	13	0	602380	571420	4306540	541050	12779390	0
tr Q9HBB3 Q9HBB3_HUMAN;tr Q8TBK	13	0	580620	1079530	684170	1001436	97757600	3439190
sp Q12788 TBL3_HUMAN;tr J3KNP2 J3	13	0	537780	195970	4632428	539050	4500300	90362

tr B3KTM6 B3KTM6_HUMAN;tr A2RUN	13	0	253042	369220	696200	0	34397000	750313
tr Q6IRT1 Q6IRT1_HUMAN;tr Q6FI45 Q	13	0	239450	512560	2738800	0	4402500	0
tr Q9GZV0 Q9GZV0_HUMAN;tr A0A02	13	0	175456	731140	753440	0	3890050	485100
sp P61011-2 SRP54_HUMAN;tr G3V4F	13	1	0	0	0	0	646830	0
tr Q8WVZ56 Q8WVZ56_HUMAN;tr Q5U0I	13	1	0	0	0	0	406210	82752
tr V9HWC7 V9HWC7_HUMAN;sp P300	13	0	0	531140	458580	516572	2565300	2902310
sp P06702 S10A9_HUMAN;tr B2R4M6	12	0	450728100	11618800	30893100	32009600	78376600	10658500
sp P35251-2 RFC1_HUMAN;sp P35251	12	0	93934000	2641569	28389800	711416	37522600	678430
tr F8VY35 F8VY35_HUMAN;sp P55209	12	0	37118170	8694776	63837221	13570959	78842290	26806787
tr B7Z6P1 B7Z6P1_HUMAN;tr A8K3K1	12	0	30985780	10858300	35127250	14484840	13603890	4022580
tr Q9HB00 Q9HB00_HUMAN;sp Q0855	12	0	30366600	23926800	21073500	25087200	18519600	21864000
tr A8K4Z4 A8K4Z4_HUMAN;tr A0A024	12	0	25988500	34303290	61111760	19551200	468044600	74615100
tr Q53G58 Q53G58_HUMAN;tr B3KN06	12	0	12938006	2559500	18248904	2409200	35538010	5112899
tr A0A024QYY3 A0A024QYY3_HUMAN;	12	0	12478000	669210	23715440	3928595	47724950	6408495
tr B0ZBD0 B0ZBD0_HUMAN;sp P39019	12	0	11876137	14064230	26662255	10262820	124281940	19511620
tr Q53GS8 Q53GS8_HUMAN;sp Q9H3P	12	0	11566000	2583338	25481000	2831500	38509290	162470
sp P29692-3 EF1D_HUMAN;tr Q9BW34	12	0	10787110	8991120	21760170	6887230	83413130	14918507
tr J3QT28 J3QT28_HUMAN;sp O43684	12	0	10249190	1229400	15985160	4632174	49818400	9866158
sp Q15084-3 PDIA6_HUMAN;sp Q1508	12	0	9405700	6098224	21272720	5479374	8602360	8388000
tr A0A087VYY6 A0A087VYY6_HUMAN	12	0	8621900	3748280	3376880	4105340	4609600	4841620
tr A0A024R845 A0A024R845_HUMAN;s	12	0	8367390	8259525	16982952	5862638	24570000	2220352
tr A0A024R994 A0A024R994_HUMAN;s	12	0	8127180	1468525	13331192	2704542	25934317	3464370
tr Q6FHX6 Q6FHX6_HUMAN;sp P3974	12	0	7700372	2214500	14722284	2086900	71183930	5077150
sp Q0VDF9 HSP7E_HUMAN;tr H7C2A1	12	0	7154900	1402100	8665900	699980	24004000	1977700
sp P29692 EF1D_HUMAN;tr D3DWK1	12	0	6116890	5941821	12113180	4281440	31573440	7348881
tr B4DE91 B4DE91_HUMAN;sp P49189	12	0	5823030	239670	12860388	1296080	23654000	1382200
sp O00629 IMA3_HUMAN;tr H7C4F6 H	12	0	5129800	1155200	4238400	2006100	26668950	5501900
tr B4DQ50 B4DQ50_HUMAN;tr B4DRV	12	0	5118880	2544650	2945260	3543130	3307100	2334210
tr A0A024R2M7 A0A024R2M7_HUMAN	12	0	5019477	1225400	13077000	1693100	9515800	1371400
sp P10155-3 RO60_HUMAN;sp P10155	12	0	4847160	0	8926210	359150	17863680	1130700
sp Q68E01-2 INT3_HUMAN;sp Q68E01	12	0	4808940	0	1566500	340680	12990000	121120
tr Q53GW1 Q53GW1_HUMAN;sp Q8W	12	0	4594200	779850	12453000	1003300	22247000	0
sp P62917 RL8_HUMAN;tr E9PKZ0 E9F	12	0	4587410	4312570	14084060	1744020	118600790	11889190
tr V9HWC2 V9HWC2_HUMAN;sp Q994	12	0	4557380	3328101	4714410	2648760	27579850	16775120
sp O60343-2 TBCD4_HUMAN;sp O6034	12	0	4538292	546080	1786700	528280	7537700	629830
tr A0A087WTA5 A0A087WTA5_HUMAN	12	0	4403999	155470	2197600	0	23933000	622790
sp Q9Y6G9 DC1L1_HUMAN;tr Q6MZE7	12	0	4375200	737040	11148090	3684000	12096000	4280777
sp Q96T76-9 MMS19_HUMAN;sp Q96T	12	0	4302900	302520	3440300	253410	11209000	774250
tr U3KQC1 U3KQC1_HUMAN;sp Q9BV	12	0	4177800	247940	6968200	1108300	22393000	1797800
sp P21281 VATB2_HUMAN;tr Q59HF3	12	0	4066050	2206100	8002200	1241800	4375000	639912
sp P10768 ESTD_HUMAN;tr X6RA14 X	12	0	4046140	5666100	7169592	3422400	87496000	4630197
sp O00116 ADAS_HUMAN;tr B7Z3Q4 I	12	0	3938800	1403000	13697000	1291500	7314600	0
tr V9HWG9 V9HWG9_HUMAN;tr B2R9	12	0	3870690	4682287	10046040	3395442	49078341	8752510
sp Q9H0D6-2 XRN2_HUMAN;tr B4DZC	12	0	3848000	1311300	2638500	508860	11622940	1475100
sp Q8NFD5 ARI1B_HUMAN;sp Q8NFD	12	0	3677400	87509	2237774	45285	6734100	168150
sp Q86UP2-2 KTN1_HUMAN;sp Q86UF	12	0	3659900	0	767450	0	0	0
tr Q75MT5 Q75MT5_HUMAN;sp P3525	12	0	3640958	213760	8422150	760470	22148820	1697300
tr Q05DH1 Q05DH1_HUMAN;sp O1481	12	0	3561430	1205590	2296700	1051382	21311000	3709080
tr Q0P607 Q0P607_HUMAN;sp O95104	12	0	3405900	55459	1140000	860090	20576000	499910
sp Q96T37-4 RBM15_HUMAN;tr A0A08	12	0	3373400	583610	2007800	0	16864000	257700
sp Q13136-2 LIPA1_HUMAN;sp Q1313	12	0	3262600	406950	338500	87785	21354520	159590
sp Q96PK6 RBM14_HUMAN;tr B4DNG	12	0	3238992	1406800	7560600	1287900	20818000	946100
sp Q86XP3 DDX42_HUMAN;tr A0A0A0	12	0	3014900	279540	712480	0	6978000	770080
sp P22392-2 NDKB_HUMAN;tr Q32Q11	12	0	2917710	7250260	5678260	4376770	14820000	35208910
sp Q13617 CUL2_HUMAN;tr A0A0A0M	12	0	2663600	270280	5563299	354980	13938110	673830
sp P49643 PRI2_HUMAN;tr A8K7A0 A	12	0	2622700	0	2900600	291930	11723000	1419070
tr A0A024R482 A0A024R482_HUMAN;s	12	0	2451100	2560000	9813000	2943600	13438000	4587900
sp Q02543 RL18A_HUMAN;tr MOR3D6	12	0	2310100	2749174	8955910	1119659	100621220	5947144
sp O60664-4 PLIN3_HUMAN;sp O6066	12	0	2261200	685390	1052800	0	17683000	7006100
sp Q6P1J9 CDC73_HUMAN;tr B4DV47	12	0	2125520	301000	582610	314270	10431300	245390
tr Q5RLJ0 Q5RLJ0_HUMAN;tr Q549M8	12	0	2030750	3364100	4012590	2641000	59354500	5481600
tr B4DS37 B4DS37_HUMAN;sp O14976	12	0	2003600	267380	745350	688700	19255000	1851730
tr Q6NX51 Q6NX51_HUMAN;sp Q96A6	12	0	1960100	525870	1278100	103580	8945700	209655
tr Q6DHZ8 Q6DHZ8_HUMAN;tr B2RBM	12	0	1946700	97795	1225000	302480	11789000	315140
tr Q96FS1 Q96FS1_HUMAN;sp O60716	12	0	1934100	0	1640200	0	8818424	381720
sp Q96FW1 OTUB1_HUMAN;tr J3KR44	12	0	1876200	3084600	9900200	3054566	53222000	4946500
sp Q14671-2 PUM1_HUMAN;sp Q1467	12	0	1817960	87854	2594790	0	19573290	441060
sp Q12873-2 CHD3_HUMAN;sp Q1287	12	0.000699	1797490	1511510	175500	964940	0	548689
sp Q1KMD3 HNR12_HUMAN;tr H3BQZ	12	0	1762800	0	1097100	0	6714400	721060
sp P11908 PRPS2_HUMAN;sp P11908	12	0	1691800	2335300	4101610	913890	29326000	1801760
tr G3XAM7 G3XAM7_HUMAN;tr B4E2C	12	0	1488876	350750	3014500	132460	12438720	861930
tr Q96F88 Q96F88_HUMAN;sp Q99575	12	0	1465100	0	924700	0	4774530	549390
sp Q9H0B6 KLC2_HUMAN;sp Q9H0B6	12	0	1236000	165540	1457000	91759	7630060	202820
sp Q9H9Y6 RPA2_HUMAN;sp Q9H9Y6	12	0	1113300	86867	1045200	0	9890595	475320
sp P07741 APT_HUMAN;sp P07741-2	12	0	1096870	1791100	1788600	742894	3163000	8309900



tr V9HWF2 V9HWF2_HUMAN;sp P409	12	0	1059217	803020	335350	0	794790	2419571
tr B7ZKT9 B7ZKT9_HUMAN;sp Q2M388	12	0	989340	0	734960	0	16828000	0
tr E7EQ69 E7EQ69_HUMAN;sp Q9GZZ1	12	0	906480	645620	2948700	460830	23506000	1500200
tr Q6UQL6 Q6UQL6_HUMAN;sp O75795	12	0	731330	601890	960450	0	21966000	377740
tr Q53HG7 Q53HG7_HUMAN;sp Q1424	12	0	635160	0	930900	0	1433700	151210
tr Q9UNF3 Q9UNF3_HUMAN;tr F5H1D	12	0	559860	0	214360	0	4294100	0
tr E7EVJ5 E7EVJ5_HUMAN;sp Q96F07-	12	0	559490	0	0	0	2314200	0
tr Q53G71 Q53G71_HUMAN;tr V9HW8	12	0	467080	84584	0	0	313600	1170680
tr A0A024R5M9 A0A024R5M9_HUMAN	12	0	419420	0	737414	0	2083500	0
tr A0A024RAE1 A0A024RAE1_HUMAN;	12	0	232331	479050	864120	165699	16543000	265030
sp Q92974-3 ARHG2_HUMAN;sp Q929	12	0	184370	0	483110	0	10532720	112610
sp Q07955 SRSF1_HUMAN;tr J3KTL2 J	12	0	0	0	271120	0	12129000	1573900
tr A0A024RDQ0 A0A024RDQ0_HUMAN	12	0	0	0	0	0	0	521800
tr H9KV75 H9KV75_HUMAN;tr B7Z565	12	0	0	0	0	0	0	48229
sp P31946-2 14338_HUMAN;tr V9HWC	12	0	0	261270	518780	0	4315500	1209900
tr V9HWB5 V9HWB5_HUMAN;sp Q151	12	0	0	140900	140280	150130	0	2059107
sp P12081-4 SYHC_HUMAN;sp P12081	12	0	0	0	0	177033	0	388504
tr Q5T081 Q5T081_HUMAN;sp P18754	11	0	34673180	16691795	63887000	10334500	80713440	12733075
tr A8K4W5 A8K4W5_HUMAN;sp Q98V	11	0	26556380	70142	45675355	5664600	55250100	5887500
sp P26368-2 U2AF2_HUMAN;sp P2636	11	0	21508000	3227400	38151000	6482900	71456000	8669939
tr Q6LET3 Q6LET3_HUMAN;sp P00492	11	0	21480220	11716450	50343570	8243830	60750570	14071681
sp P05089 ARG1_HUMAN;sp P05089-	11	0	17645980	5140280	4464280	7657900	2281370	3032400
tr A0A024QZF1 A0A024QZF1_HUMAN;	11	0	13022210	5602220	32039100	6844994	52888000	4484600
tr V9HW12 V9HW12_HUMAN;sp P321	11	0	11131900	6395610	26046360	5777830	22003530	9820700
tr A0A024R9D2 A0A024R9D2_HUMAN;	11	0	10016072	3774665	6882200	1408608	725880	0
tr B4DM97 B4DM97_HUMAN;sp P6116	11	0	8962600	2540300	16659000	4172800	27001000	9711000
sp Q9UKX7-2 NUP50_HUMAN;tr A0A0	11	0	8097100	2202500	23618915	3666100	37669132	4662500
sp P38159 RBMX_HUMAN;sp P38159-	11	0	7966919	1378900	19098160	592920	61683900	3629105
tr A0A024R6S1 A0A024R6S1_HUMAN;s	11	0	7113000	3349400	14835000	1752200	27982000	9292230
tr Q8NCK5 Q8NCK5_HUMAN;tr A0MN	11	0	6657200	1740500	10672000	1419600	33291000	4453710
sp P40429 RL13A_HUMAN;tr Q9BSQ6	11	0	6580700	1911780	11984760	1438190	176494600	10681660
sp Q68EM7-6 RHG17_HUMAN;sp Q68E	11	0	6175600	410380	2172400	0	7753540	0
sp Q95336 6PGL_HUMAN;tr MOR261 F	11	0	5987140	1411700	10191000	1340178	60211000	2868556
tr E7EN20 E7EN20_HUMAN;tr A0A024	11	0	5894700	736570	3703319	375180	19087000	1685100
tr Q53Z07 Q53Z07_HUMAN;sp P32969	11	0	5487520	5672800	12547000	3029810	250807290	11709750
tr B4E1E2 B4E1E2_HUMAN;sp O14964	11	0	5255100	673820	2891500	113300	15255220	694700
tr B7Z4K8 B7Z4K8_HUMAN;tr E7ETZ4	11	0	5148200	1491500	8481800	982060	28522000	1002528
sp Q15813 TBCE_HUMAN;sp Q15813-2	11	0	5089200	0	8261250	1700700	18306580	952280
sp Q9BY77 PDIP3_HUMAN;tr F6VRR5	11	0	4636300	1192200	7133100	1098000	27772000	2563640
tr A0A087WUK2 A0A087WUK2_HUMAN	11	0	4475550	4238173	11017230	2812540	86388238	7523770
tr B4DQT0 B4DQT0_HUMAN;tr B4E1J2	11	0	4311000	606360	7380800	267600	24863000	1480600
sp P56545 CTBP2_HUMAN;tr Q5SQP8	11	0	4168300	1441392	8899470	601920	21164150	1743500
sp P98175-2 RBM10_HUMAN;sp P9817	11	0	4015300	634820	1810800	141760	11788000	1664100
sp O15294 OGT1_HUMAN;sp O15294-	11	0	3932288	538710	2897800	516320	5100370	539690
sp P55263-2 ADK_HUMAN;sp P55263	11	0	3910000	731940	5299300	1384368	7095200	1951700
tr Q2L6I0 Q2L6I0_HUMAN;sp Q96QC0	11	0	3880200	1546200	1861700	878610	17395200	700190
sp P62750 RL23A_HUMAN;tr H7BY10	11	0	3507490	280400	9634010	2123310	126858500	6240030
sp P62280 RS11_HUMAN;tr M0QZC5 F	11	0	3506110	2012000	13506211	618860	110338160	5173568
sp Q15907 RB11B_HUMAN;tr H3BMH2	11	0	3376370	5005100	11860200	4061500	8614870	3204300
tr A8K5D9 A8K5D9_HUMAN;sp Q9NQ1	11	0	3316900	82122	1269300	0	3305500	0
sp Q52U0-2 FA98B_HUMAN;sp Q52U0	11	0	3314220	1832844	10345860	1432103	28839100	4869560
sp P31947 1433S_HUMAN;sp P31947-2	11	0	3086069	1690640	0	0	9762890	3974400
sp Q9Y383 LC7L2_HUMAN;sp Q9Y383-	11	0	3024800	356820	5541800	156160	10276000	2064700
tr B4E3E6 B4E3E6_HUMAN;tr B4DDB6	11	0	2859614	585994	4637008	592848	36396000	4568770
tr Q6IAU5 Q6IAU5_HUMAN;tr B2R665	11	0	2840382	3076834	18215000	678820	16195000	604420
sp Q99661 KIF2C_HUMAN;sp Q99661-	11	0	2583500	1254951	5151052	4072110	15276730	5983401
sp Q9Y5K5-2 UCHL5_HUMAN;sp Q9Y5	11	0	2562980	0	3563200	2106700	29953000	5591780
sp O43491 E41L2_HUMAN;sp O43491-	11	0	2560100	185434	1133700	0	4731000	156650
sp Q96EV2 RBM33_HUMAN;sp Q96EV	11	0	2375700	0	2755150	0	12879000	208850
tr Q53HS1 Q53HS1_HUMAN;sp Q9NR0	11	0	2331300	0	5560570	0	3447200	0
tr A0A087X0Q1 A0A087X0Q1_HUMAN;	11	0	2277400	2780900	4306700	2808400	20775000	2330100
sp Q5T6F2 UBAP2_HUMAN;tr B4DRB6	11	0	2185900	304508	1839600	405160	12207000	0
sp Q9NRY5 F1142_HUMAN;tr E7ESJ7 F	11	0.00037	2135600	0	0	327270	4749100	1270300
tr B2R7T8 B2R7T8_HUMAN;sp P47756-	11	0	2126130	7428454	3853990	1536336	30193770	6206430
tr Q4ZG72 Q4ZG72_HUMAN;tr A0A024	11	0	2074300	217920	3826400	0	22444000	366121
tr A0A024R534 A0A024R534_HUMAN;s	11	0	1704136	0	1133100	0	6428160	801924
sp Q15654 TRIP6_HUMAN;tr F2ZC06 F	11	0	1643500	299330	6057200	284710	12073610	480655
tr V9HWI0 V9HWI0_HUMAN;sp P1455	11	0	1443210	316980	983880	0	7738920	2814360
sp Q13561 DCTN2_HUMAN;sp Q13561	11	0	1270800	214580	7059700	352900	20992000	5790800
tr Q5TZZ9 Q5TZZ9_HUMAN;sp P04083	11	0	1236180	1606150	1902520	2146760	21625520	5343860
sp P48382-2 RFK5_HUMAN;tr F8W689	11	0	1117000	506930	2059100	0	16271395	0
tr A0A087VWW7 A0A087VWW7_HUMAN	11	0	1101800	201300	1485620	0	11378360	0
sp P60900 PSA6_HUMAN;tr G3V5Z7 G	11	0	1011770	644260	714500	680130	3600970	2956170
tr B4DYK6 B4DYK6_HUMAN;tr A0A024	11	0	949940	0	0	312340	4961000	0
sp O43660 PLRG1_HUMAN;sp O43660-	11	0	918390	305180	3502900	331010	12106000	265930

sp Q6NZY4 ZCHC8_HUMAN;tr A8K559	11	0	901500	0	774210	0	20024000	641950
tr B4DS79 B4DS79_HUMAN;tr B4DKH5	11	0.000364	862540	0	0	234050	0	0
tr A0A024R9G4 A0A024R9G4_HUMAN;	11	0	719290	1151300	2762170	1386300	32409000	2550800
tr A1L3A7 A1L3A7_HUMAN;sp Q7Z417	11	0	660182	0	11465154	176000	20594370	915810
tr D5MQE1 D5MQE1_HUMAN;tr B2RE5	11	0	633300	148020	1534900	178700	17737000	1015000
sp Q9NXF1-2 TEX10_HUMAN;tr A0A02	11	0	591240	0	688570	0	13251000	0
sp Q9HCK8 CHD8_HUMAN;sp Q9HCK8	11	0	468850	0	233530	0	9851100	0
sp Q9NYV4-2 CDK12_HUMAN;sp Q9N	11	0	428350	0	573360	0	10713000	0
sp Q86W42 THOC6_HUMAN;sp Q86W	11	0	421500	178360	696480	0	18361000	1065450
sp Q13257 MD2L1_HUMAN;sp Q13257	11	0	411520	0	791470	375570	30210990	369360
tr E9PC74 E9PC74_HUMAN;sp Q13144	11	0	384950	458430	703190	0	18523650	2381540
tr Q6FGU2 Q6FGU2_HUMAN;sp P2391	11	0	263660	1896100	1643930	756590	21740270	913150
tr Q6FGS1 Q6FGS1_HUMAN;sp O4339	11	0	238840	242240	712800	0	3542200	489670
sp P61981 I433G_HUMAN;tr B3KNB4	11	0	225980	0	1834783	7288050	2962100	24357100
tr D9IAI1 D9IAI1_HUMAN;sp P30086	11	0	204070	618340	118750	177490	732570	1649382
sp Q9GZS3 WDR61_HUMAN;tr H0YN8	11	0	187330	408180	1809800	0	22715560	1472200
tr B4DHS5 B4DHS5_HUMAN;sp Q9NXH	11	0	167170	0	1541400	0	12189000	306680
sp Q96EE3 SEH1_HUMAN;sp Q96EE3-1	11	0	158820	111040	1167300	146630	14172930	314780
sp Q04760-2 LGUL_HUMAN;tr X5DNM	11	0	65656	175980	676610	115420	4964400	3469579
sp O14979-3 HNRDL_HUMAN;sp O149	11	0.000693	0	0	199200	0	1607200	0
sp Q92625 ANS1A_HUMAN;tr Q49AR9	11	0	0	0	287010	0	9597800	0
sp Q9UPN9-2 TRI33_HUMAN	11	0.000355	0	0	0	0	365430	0
tr B4DHY7 B4DHY7_HUMAN	11	1	0	0	0	0	3492700	0
tr B4DI68 B4DI68_HUMAN;tr G5E9S2	11	1	0	0	0	0	541700	0
tr Q68E05 Q68E05_HUMAN;sp O43399	11	1	0	0	0	0	0	0
tr Q86U75 Q86U75_HUMAN;tr A9CQZ	11	1	0	0	0	0	0	0
tr J3QSV6 J3QSV6_HUMAN;tr A0PJ61	11	0	0	0	1108500	0	18626460	421071
sp Q15019 SEPT2_HUMAN;sp Q15019-	11	0	0	0	0	0	0	686620
sp O00429-4 DNM1L_HUMAN;sp O004	11	0	0	94268	1040900	0	1203500	132004
tr B2R6U8 B2R6U8_HUMAN;tr A0A024	11	0	0	381230	525030	0	4956100	1659900
sp Q55J5 HP1B3_HUMAN;tr X6RGJ2	11	0	0	294930	2441770	162680	25345300	1445270
tr Q05CN7 Q05CN7_HUMAN;sp Q9UK	11	0	0	910420	2294540	1066100	10266100	1571800
sp P28074 PSB5_HUMAN;sp P28074-3	11	0	0	0	122620	141740	522530	672150
tr F8W543 F8W543_HUMAN	11	1	0	0	0	153680	0	0
tr B4DVJ0 B4DVJ0_HUMAN;tr K7EQ48	11	0	0	140830	0	145630	0	2648210
sp P05109 S10A8_HUMAN	10	0	292375100	13296300	28627600	34376900	47099600	15969200
sp P04632 CPN51_HUMAN;tr K7ELJ7	10	0	45288340	10646830	78166000	6810900	57251000	15659376
tr Q96HT3 Q96HT3_HUMAN;sp Q1516	10	0	41290900	125930	33595500	16735700	47467000	50980000
tr A0A024R3Q3 A0A024R3Q3_HUMAN;	10	0	29106820	41876370	73948710	21673918	169311550	35314410
sp P62249 RS16_HUMAN;tr M0R210 N	10	0	26782870	11893570	40191890	9130680	292052500	19417610
sp P61160 ARP2_HUMAN;sp P61160-2	10	0	9953806	0	20234280	3599800	24946520	2604084
sp Q96P63 SPB12_HUMAN;sp Q96P63	10	0	8467900	7142200	7084500	10292900	5541500	5757010
sp Q8IWX8 CHERP_HUMAN;tr J3QK89	10	0	8318100	1287800	4307500	541870	27433240	1878500
tr F2Z2V0 F2Z2V0_HUMAN;sp Q99829	10	0	7588700	839749	13687000	2665180	42234830	7175330
tr A8K5J1 A8K5J1_HUMAN;sp P11172	10	0	7460100	1851100	17354000	2051600	24876000	4842799
tr Q5JR94 Q5JR94_HUMAN;sp P62241	10	0	7270860	4771020	18445090	3302100	252955000	12329270
sp P35659 DEK_HUMAN;sp P35659-2	10	0	6824800	4116951	12684000	2943700	20804140	7048610
sp P11216 PYGB_HUMAN;tr Q59GM9	10	0	6316600	2095600	8044600	816990	24166200	253550
tr Q6NZ55 Q6NZ55_HUMAN;tr A8K4C	10	0	5950750	3718710	17443970	1159890	200841550	4658020
sp Q09028-3 RBBP4_HUMAN;sp Q090	10	0	5857700	2117500	6787500	538870	18789480	3839950
sp P18085 ARF4_HUMAN;tr C9JPM4 C	10	0	5750810	8550300	9328600	4869200	28111000	7419700
sp P51149 RAB7A_HUMAN;tr C9J592	10	0	5397040	6415100	8486740	2817780	12818000	2457080
sp Q96SN8-3 CKSP2_HUMAN;tr B9EG7	10	0	5260400	0	2225577	0	1418200	0
tr A6NJA2 A6NJA2_HUMAN;sp P5457	10	0	5217700	829510	2155700	1603300	15532000	3855714
tr A0A024R9J0 A0A024R9J0_HUMAN;sp	10	0	5120500	146206	2295200	0	16549000	1373100
tr A0A087WSW7 A0A087WSW7_HUMA	10	0	5031600	920660	3543750	485540	13355010	390990
sp P52306 GDS1_HUMAN;sp P52306-5	10	0	4814680	1554762	11621000	2120176	14218200	1663966
tr A0A024RAD5 A0A024RAD5_HUMAN	10	0	4686200	164620	8475600	199760	1063400	0
tr D6W625 D6W625_HUMAN;sp Q131	10	0	4278800	762150	771310	423870	7821500	218740
tr B2RCJ6 B2RCJ6_HUMAN;sp Q9BZH6	10	0	4046170	118740	3208520	0	6276510	497570
tr A0A024R1U4 A0A024R1U4_HUMAN;	10	0	3925856	7219680	15932587	5939750	9084700	3887780
sp P43034 LIS1_HUMAN;tr B4DF38 B4	10	0	3836170	1068873	6811800	960310	29462250	3468901
sp Q7Z3U7-2 MON2_HUMAN;tr B7ZM	10	0	3811200	0	1071300	741730	4892100	0
tr E7EVX8 E7EVX8_HUMAN;tr F1T0A5	10	0	3626000	361610	8870800	859950	28272540	347760
sp Q9UI26 IPO11_HUMAN;sp Q9UI26-	10	0	3530700	0	3043830	0	12384000	406110
tr Q5BKZ2 Q5BKZ2_HUMAN;sp P5229	10	0	3312100	257570	8681100	663980	19665000	1880900
sp P05023-3 AT1A1_HUMAN;tr B7Z3V	10	0	3177820	127310	1413220	0	0	0
tr B1AKK2 B1AKK2_HUMAN;sp O9476	10	0	3133000	2087600	3719100	737180	60967000	2914800
sp Q99729-3 ROAA_HUMAN;tr D6R9P	10	0	3113190	1057888	4959610	1041710	11291350	4195520
sp Q6P4R8-3 NFRKB_HUMAN;tr A0A0	10	0	3091400	254530	682420	0	4121102	0
sp O00268 TAF4_HUMAN;tr V9GY14 V	10	0	2886200	206660	2059600	0	9941100	274810
tr I3LOH8 I3LOH8_HUMAN;tr B4DS24 E	10	0	2801103	1222670	4091900	1037300	15021740	1510000
tr F6T1Q0 F6T1Q0_HUMAN;sp Q6L8Q	10	0	2676300	0	3870000	254430	12746000	0
tr Q8TDR3 Q8TDR3_HUMAN;tr Q8NEH	10	0	2632400	0	2305400	0	11865970	452930
tr F8W8R3 F8W8R3_HUMAN;sp P4900	10	0	2391040	1059752	1713400	299060	12641000	1434900

tr B4DJU4 B4DJU4_HUMAN;tr A0A024	10	0	2322410	1886233	4075400	1163600	17165340	2484966
sp Q95394 AGM1_HUMAN;sp O95394-	10	0	2288145	0	5654959	0	18371220	0
sp Q9NR09 BIRC6_HUMAN;tr Q9H8B7	10	0	2276300	0	68790	92529	4564500	0
tr M0R3F6 M0R3F6_HUMAN;sp Q8IX0	10	0	2270000	0	792160	0	9937000	0
sp Q14684 RRP1B_HUMAN;sp Q14684	10	0	2227030	1069600	1557190	597040	18321700	1207350
tr Q5TDG3 Q5TDG3_HUMAN;sp Q9UN	10	0	2196720	0	604670	0	4388760	0
sp P42345 MTOR_HUMAN;tr B3KX59	10	0	2172400	432450	356650	0	8517500	0
sp Q99996-5 AKAP9_HUMAN;sp Q999	10	0	2149300	0	661120	0	1462100	0
sp Q66K74-2 MAP15_HUMAN;tr A8K9	10	0	1847900	462907	1162000	0	11888000	520950
tr C9JFE4 C9JFE4_HUMAN;tr A0A096L	10	0	1834400	349050	6914400	618170	22124100	648880
sp O43324 MCA3_HUMAN;sp O43324-	10	0	1695008	3012100	4374338	1334700	30896580	5352881
sp Q9NZJ4 SACS_HUMAN;sp Q9NZJ4-	10	0	1653900	0	356110	0	2815900	0
tr B7ZM87 B7ZM87_HUMAN;tr A2RUF	10	0	1630700	296200	1225110	0	5804100	145400
tr B4DL80 B4DL80_HUMAN;tr G5EA36	10	0	1556400	191330	2491600	0	11362000	614360
sp O43747 AP1G1_HUMAN;tr Q8IY97	10	0	1540990	440590	3840190	579680	18107800	977840
tr B1AJY7 B1AJY7_HUMAN;sp O75832	10	0	1516100	1964835	5214612	1496300	38922220	1307100
sp Q8IZH2-2 XRN1_HUMAN;sp Q8IZH	10	0	1306600	0	277380	0	7451000	0
sp Q9UBQ5 EIF3K_HUMAN;sp Q9UBQ	10	0	1074600	1568500	3040800	977370	4683500	1996400
tr Q5JW30 Q5JW30_HUMAN;sp O9579	10	0	1044900	429320	1057300	215810	7641000	139000
sp P42025 ACTY_HUMAN;tr Q13841 C	10	0	1033800	0	4385200	0	8199000	1050700
sp Q8TBC4-2 UBA3_HUMAN;tr B2RBP	10	0	935460	3205630	2045600	3939490	5873370	1413766
tr B4DWS6 B4DWS6_HUMAN;sp Q53G	10	0	839060	641830	2949200	505810	467510	0
tr B4E074 B4E074_HUMAN;tr B4E303	10	0	805330	0	2749700	0	6848700	331060
sp Q9Y5Q8-2 TF3C5_HUMAN;sp Q9Y5	10	0	801210	0	1084700	182270	10895000	85070
tr D6W646 D6W646_HUMAN;sp Q6ZS	10	0	777850	0	251250	0	10908000	0
sp Q9Y6D9 MD1L1_HUMAN;tr A4D218	10	0	710580	135420	2084200	295240	18550000	6757000
sp Q9NYF8-2 BCLF1_HUMAN;sp Q9NY	10	0	701070	770240	155520	127990	12799000	707740
tr A0A024R6H0 A0A024R6H0_HUMAN;	10	0	699580	263240	391650	281040	11053000	124350
tr A0A024R7F7 A0A024R7F7_HUMAN;s	10	0	655725	236560	237190	0	5107700	109310
tr A0A024RCC9 A0A024RCC9_HUMAN;	10	0	541250	700440	2864900	405700	2664100	1764600
tr HOYMZ1 HOYMZ1_HUMAN;tr HOYL6	10	0	531100	458071	1220160	1057907	4501800	1993540
sp Q9UIX2 CDC23_HUMAN;sp Q9UIX2	10	0	438930	0	1700900	0	3002890	0
sp P25786 PSA1_HUMAN;sp P25786-2	10	0	385420	0	0	0	12912000	136940
sp Q9NTK5 OLA1_HUMAN;tr J3KQ32	10	0	379180	0	0	0	0	406520
tr Q0QF37 Q0QF37_HUMAN;tr Q75MT	10	0	373120	140060	0	0	0	0
tr A0A087WWU8 A0A087WWU8_HUM	10	0	338200	196570	161690	153910	0	849130
tr A0A087X0I4 A0A087X0I4_HUMAN;sp	10	0	335460	370340	404350	0	22729000	566020
tr A0A024R0F1 A0A024R0F1_HUMAN;s	10	0	283580	0	747310	0	9896000	0
sp Q9NQG5 RPR1B_HUMAN;tr E9PIQ9	10	0	272970	710760	626334	282990	11960000	1149370
tr B3KY04 B3KY04_HUMAN;sp Q96CX2	10	0	269780	209160	5669900	0	11518010	811960
tr A0A024R896 A0A024R896_HUMAN;s	10	0	259880	201940	1750700	1205700	15815000	16584000
tr B3KXN0 B3KXN0_HUMAN	10	1	242440	0	623260	0	389453	0
sp Q16222-2 UAP1_HUMAN;sp Q1622	10	0	0	0	0	0	867610	0
sp Q5TSY3-2 CAMP1_HUMAN;sp Q5TS	10	0	0	0	217640	0	10470000	0
sp Q9UMR2-2 DD19B_HUMAN;tr Q53C	10	0	0	0	1155500	0	2609400	0
tr A0A024R753 A0A024R753_HUMAN;s	10	0	0	0	95389	0	12495000	0
tr B4DM78 B4DM78_HUMAN;tr Q1434	10	0	0	0	266300	0	553270	0
tr H0Y3K4 H0Y3K4_HUMAN;tr B4DVQ2	10	1	0	0	0	0	1188800	0
sp Q92888-2 ARHG1_HUMAN;tr A0A0	10	0	0	0	0	0	7581000	168440
tr A8K8X0 A8K8X0_HUMAN;sp Q14CX	10	0	0	0	0	0	0	161230
tr A0A024RBE7 A0A024RBE7_HUMAN;s	10	0.005178	0	106010	160250	0	0	0
sp Q96AC1-2 FERM2_HUMAN;tr H0YJ	10	0	0	163930	0	0	4721500	282100
tr A8K3D0 A8K3D0_HUMAN;tr B4DUD	10	0	0	125340	362619	64356	324440	668679
tr Q53T99 Q53T99_HUMAN;sp Q9GZL7	10	0	0	160530	1395300	250890	8475500	204500
sp P01857 IGHG1_HUMAN;tr Q6PYX1	9	0	345194000	305691000	356642900	291049000	298365000	459413000
tr V9HW43 V9HW43_HUMAN;sp P047	9	0	62132000	3402120	5122800	5710370	16264000	4247900
tr Q5U0I6 Q5U0I6_HUMAN;sp P62820	9	0	23949570	6690178	43212760	3745730	24861320	4099883
tr B2R4R0 B2R4R0_HUMAN;sp P62805	9	0	18786530	8181711	23778280	2842941	66231210	15882550
tr B4E0K0 B4E0K0_HUMAN;tr Q5JRG1	9	0	17968593	10121863	48073240	6563000	38429000	1799900
tr V9HWE9 V9HWE9_HUMAN;sp P092	9	0	13532270	7518862	18792720	4055850	80146270	13825320
sp Q58FF8 H90B2_HUMAN	9	0	12005840	4549400	21521150	5923100	18960640	4563860
sp POCW22 RS17L_HUMAN;sp P08708	9	0	11561890	4525790	24901800	3552870	154351206	15897140
tr X5D8S6 X5D8S6_HUMAN;tr Q71UA4	9	0	9395650	1888000	10577569	1568500	34966657	2943627
tr H9ZYJ2 H9ZYJ2_HUMAN;sp P10599	9	0	8393900	7196100	4784730	4642230	12220200	40153100
tr Q96DV6 Q96DV6_HUMAN;tr A2A3R	9	0	8314050	337380	21152830	421661	166110000	3118850
tr A0A087VXS7 A0A087VXS7_HUMAN	9	0	7814900	0	25354000	4184085	18281000	3910400
tr B2RD27 B2RD27_HUMAN;sp P51665	9	0	7775657	0	7778100	1995400	31386291	10380620
sp O00154-4 BACH_HUMAN;sp O0015	9	0	7050790	487610	11837932	1003462	29468670	2284863
tr B2R7X3 B2R7X3_HUMAN;sp Q13112	9	0	6914200	11243520	10422900	96466	8328820	139860
tr F5GX71 F5GX71_HUMAN;tr H3BPB8	9	0	6725500	2223600	12434000	953860	33538330	3383200

sp Q96PU8-9 QK1_HUMAN;sp Q96PU8	9	0	5993410	1878360	21541080	3820600	66828600	7143276
tr V9HW39 V9HW39_HUMAN;sp Q9NI	9	0	5027000	303990	10152720	1299800	29955870	1283200
tr A8K8N3 A8K8N3_HUMAN;sp Q96R0	9	0	4958500	262370	2508400	0	3813900	104340
sp Q3ZCM7 TBB8_HUMAN;tr A0A075B	9	0	4049600	0	0	1276300	16939000	0
sp P24666 PPAC_HUMAN;tr Q59EH3 C	9	0	3925470	1478200	7831850	1127500	47354000	3342800
sp O76003 GLRX3_HUMAN	9	0	3701406	480400	5281860	664480	9263530	3328740
tr A0A024R4T4 A0A024R4T4_HUMAN;s	9	0	3539378	2470700	12432000	1375088	78528600	7070900
tr Q6FHH6 Q6FHH6_HUMAN;tr Q53TN	9	0	3381548	0	4039600	690280	17674450	1498570
tr A0A024R0E5 A0A024R0E5_HUMAN;s	9	0	3300250	3216858	6725490	1953170	29093000	6350070
sp Q9Y613 FHOD1_HUMAN;tr A0A068	9	0	3274900	0	1541600	0	6285000	0
tr A0A087WXZ3 A0A087WXZ3_HUMAN	9	0	3240820	0	6886200	1323253	20299090	799291
sp Q9ULT8 HECD1_HUMAN;tr A0A087	9	0	3015900	0	1108000	543950	1785200	0
sp P35611-2 ADDA_HUMAN;tr E7EV99	9	0	2919485	507430	2156689	0	9594830	1738290
sp Q9UJY4 GGA2_HUMAN;tr B0QYR9	9	0	2810700	244370	10445450	1012100	11805180	0
sp Q9HOC8 ILKAP_HUMAN;tr F8SNU7	9	0	2803600	205530	4695100	0	9504800	951430
sp Q9P2N5 RBM27_HUMAN;tr U3KPZ7	9	0	2726100	1204700	1812700	178540	10923000	3193700
tr B4DKX4 B4DKX4_HUMAN;sp Q53EL6	9	0	2722000	401150	3515000	295640	4686600	2494100
tr B7Z8A2 B7Z8A2_HUMAN;sp Q9UHG	9	0	2564200	275330	0	0	0	0
sp Q96AG4 LRC59_HUMAN;tr I3L223 I	9	0	2542380	3803050	10267411	1733734	4793400	0
sp O95487-2 SC24B_HUMAN;sp O9548	9	0	2430900	284880	1230300	107200	8636932	304923
tr A0A024QZD1 A0A024QZD1_HUMAN	9	0	2349400	428340	3425270	144911	148255280	4501990
sp Q13151 ROA0_HUMAN	9	0	2202774	2832760	6935500	1121866	86599370	9382748
tr A0A087WYU1 A0A087WYU1_HUMAN	9	0	2180700	267870	3024700	0	12630000	0
sp O75150-4 BRE1B_HUMAN;tr H3BP7	9	0	2124800	175630	1199900	0	8325500	367350
tr B4DKE6 B4DKE6_HUMAN;sp P40938	9	0	2096207	93608	1619700	805956	13731590	1586068
sp Q92769-3 HDAC2_HUMAN;sp Q927	9	0	1868200	0	952700	191850	3052700	916227
tr A0A024RCU9 A0A024RCU9_HUMAN;	9	0	1794570	221834	5735620	183343	15699770	451213
sp Q9Y3Z3-4 SAMH1_HUMAN;sp Q9Y3	9	0	1791800	215190	1151600	331572	9786300	188670
tr B4DII5 B4DII5_HUMAN;sp O60684 I	9	0	1789500	0	1681600	398450	5582800	504700
tr Q0QER2 Q0QER2_HUMAN;tr V9HW.	9	0	1667451	1543500	4021600	1106900	4878560	3162038
tr B2RAU8 B2RAU8_HUMAN;sp Q1497	9	0	1643700	849224	319020	130889	12324200	347255
tr E9PGT1 E9PGT1_HUMAN;sp Q15631	9	0	1636880	1499710	3685180	1184550	11125960	2562250
tr B4DT77 B4DT77_HUMAN;tr B4DWU	9	0	1618200	546784	3179100	237430	11606690	1026050
tr A0A087WUY3 A0A087WUY3_HUMAN	9	0	1592000	298750	2178600	305230	16915440	543110
tr C9J2Z4 C9J2Z4_HUMAN;tr B4DZH9 I	9	0	1560000	313000	3797277	628007	525440	360210
sp Q9UQ13 SHOC2_HUMAN;sp Q9UQ:	9	0	1522100	379610	4059200	287720	18603000	0
sp A3KN83-3 SBN01_HUMAN;sp A3KI	9	0	1499700	515580	618030	197430	3351700	118780
sp P62633-2 CNBP_HUMAN;sp P62633	9	0	1441613	511620	1341200	382930	20291000	1879200
tr A4QPB0 A4QPB0_HUMAN;tr A0A02	9	0	1321400	0	503750	0	3549200	0
sp Q92879-5 CELFI_HUMAN;sp Q9287	9	0	1308600	1090800	7193600	1163100	18901940	2119500
tr Q59GW5 Q59GW5_HUMAN	9	0	1294930	0	2381050	0	13261500	0
sp P49721 PSB2_HUMAN;tr B7Z478 B	9	0	1258960	0	0	243677	1133700	1167700
sp Q6UVJ0 SAS6_HUMAN;tr B4DYM7	9	0	1244000	1344700	5563000	207850	6287300	184420
tr C9JNW5 C9JNW5_HUMAN;tr V9HW	9	0	1239500	1436283	5790348	793650	84989700	3198650
tr B7Z6H4 B7Z6H4_HUMAN;sp O14802	9	0	1221700	0	323200	0	1336300	0
sp Q9NR50 EI2BG_HUMAN;sp Q9NR50	9	0	1192900	132410	2617100	591960	11700000	339980
tr H0YH87 H0YH87_HUMAN;sp Q99700	9	0	1187300	187020	680020	0	10616000	288860
sp O60566-2 BUB1B_HUMAN;sp O605	9	0	1185600	0	1547600	0	1931000	0
sp O15357 SHIP2_HUMAN;tr A0A0A0A	9	0	1107700	0	1101500	154520	7431700	76688
tr B2RAG9 B2RAG9_HUMAN;sp Q1564	9	0	1083700	157100	327410	157020	9038000	298580
sp Q8WXI9 P66B_HUMAN;tr B3KSZ4 E	9	0	1072382	990900	5205100	1205500	10035490	424160
sp P46779-3 RL28_HUMAN;sp P46779	9	0	1033500	374730	3579600	212300	48049000	3338743
sp Q99543 DNJC2_HUMAN;sp Q99543	9	0	857680	1893900	1847700	1655900	16732780	3911500
sp Q9Y5Q9 TF3C3_HUMAN;tr H7BZV8	9	0	855670	146330	373950	158250	7713100	313630
tr B7WPG3 B7WPG3_HUMAN;sp Q8W	9	0	837140	0	513660	115750	4132000	468230
sp O60502-4 OGA_HUMAN;sp O60502	9	0	794420	0	0	0	7637000	0
tr A0A087X0Z0 A0A087X0Z0_HUMAN;s	9	0	786640	1864754	5336760	991910	42617620	3034800
sp Q92522 H1X_HUMAN	9	0	781273	1007380	4860850	225102	95582510	3962690
tr Q9BPW0 Q9BPW0_HUMAN;tr HOYD	9	0	742240	0	160290	0	2541400	770490
sp Q6ZRS2-3 SRCAP_HUMAN;sp Q6ZR	9	0	649970	0	184500	0	4564400	0
sp P35520 CBS_HUMAN;sp P35520-2 C	9	0	638130	0	2401000	0	2935800	3483820
sp P19105 ML12A_HUMAN;sp O14950	9	0	586940	1575545	5586600	1478900	76784000	4631383
tr Q6FGJ9 Q6FGJ9_HUMAN;sp P21266	9	0	441430	710650	2853530	250540	8283820	1857800
sp Q99426 TBCB_HUMAN;tr K7EK42 K	9	0	431970	1104000	1930400	742619	16287000	1462400
sp P23193 TCEA1_HUMAN;tr B7Z4W0	9	0	419123	173610	1285505	0	8898600	160130
tr A4D0Z3 A4D0Z3_HUMAN;sp P84085	9	0	382890	632849	1427470	673080	3652900	748060
sp Q9BZK7 TBL1R_HUMAN;tr B7Z475	9	0	375270	0	3279100	0	17761000	0
tr Q53GG3 Q53GG3_HUMAN;tr B3KN5	9	0	357410	194990	0	0	0	0
sp P09525 ANXA4_HUMAN;tr V9HW55	9	0	334720	659550	5680000	575590	4378080	365180
tr B4DWN1 B4DWN1_HUMAN;tr A8K7	9	0	330350	0	0	0	13727700	150360
sp A11070 ILVBL_HUMAN;tr MOR026 I	9	0	262410	0	103920	0	0	0
sp O95861-4 BPNT1_HUMAN;tr V9HW	9	0	244220	0	313191	162244	6976000	2380640
tr Q6IAX1 Q6IAX1_HUMAN;sp P37268	9	0	234180	0	2493200	202350	0	0
sp Q9UGI8-2 TES_HUMAN;tr B2RDR4	9	0	225410	120086	839850	0	9025700	602687
sp Q8N766-4 EMC1_HUMAN;sp Q8N76	9	0	208910	0	0	0	0	232310



tr B7Z592 B7Z592_HUMAN;sp Q8WU9	9	0	136510	0	1359600	0	11635000	959290
sp P61086 UBE2K_HUMAN;tr B4DI22 E	9	0	123120	918110	407340	163920	2639800	1140000
sp P15531 NDKA_HUMAN;sp P15531-2	9	0	91906	1608800	953760	654410	2306300	2656500
tr Q53GE2 Q53GE2_HUMAN;tr Q53GC	9	0	84163	116630	679440	0	9250300	1142730
sp O60502 OGA_HUMAN;sp O60502-3	9	0.005168	0	0	0	0	0	0
sp O60832 DKC1_HUMAN;sp O60832-2	9	0	0	0	0	0	7161500	0
tr Q4KMR3 Q4KMR3_HUMAN;sp Q129	9	0	0	0	921080	0	7350700	155630
sp P14314-2 GLU2B_HUMAN;tr A0A02	9	0	0	0	0	0	2572790	496169
sp P22061 PIMT_HUMAN;sp P22061-2	9	0	0	0	110930	0	631060	1560400
sp P51116 FXR2_HUMAN;tr I3L1Z2 I3L	9	0	0	282330	532850	0	4686700	259350
tr F8W1A4 F8W1A4_HUMAN;sp P5481	9	0	0	197590	0	0	0	784420
tr B4DRA5 B4DRA5_HUMAN;tr B4DJW	9	0	0	471290	1615700	303840	5995000	962420
tr A0A024R1K7 A0A024R1K7_HUMAN;s	9	0	0	312300	285190	169690	1978900	1550000
sp Q69YN2 C19L1_HUMAN;sp Q69YN2	9	0	0	98572	467080	388070	8505278	0
tr B4DV28 B4DV28_HUMAN;tr V9HWE	9	0	0	365240	0	486340	4382700	477890
sp P12273 PIP_HUMAN	8	0	30819300	20347800	22355600	24738200	27335700	31651800
tr V9HWI3 V9HWI3_HUMAN;sp P0733	8	0	29773560	1310840	1603220	3130210	1132570	1567530
tr Q55QT9 Q55QT9_HUMAN;tr B2R675	8	0	28614700	16315000	69308000	11355690	79246000	27063300
sp P62263 RS14_HUMAN;tr E5RH77 E	8	0	19216930	13030180	40230410	12606110	206833790	26222580
tr Q59H57 Q59H57_HUMAN;tr B4DR7C	8	0	12685770	3793913	20442636	2356959	122591000	2720940
tr B2R4W8 B2R4W8_HUMAN;sp P6224	8	0	11418860	7790608	20367398	10157740	182022150	19975400
sp P62081 RS7_HUMAN;tr B5MCP9 B5	8	0	10720100	8720006	20357600	2648310	107949430	8984770
tr B4DMT5 B4DMT5_HUMAN;sp O003	8	0	10301000	4005400	15218000	2799000	19335000	5332180
tr B7Z2Z1 B7Z2Z1_HUMAN;sp Q15424	8	0	9606100	1360900	3576300	640860	8444800	1140900
tr A8YXX4 A8YXX4_HUMAN;sp P15104	8	0	9133948	257172	738054	381056	1046560	421314
sp Q15185-4 TEBP_HUMAN;tr A0A024	8	0	7811000	2233850	9425880	3251800	82507000	6370600
tr Q6IBR8 Q6IBR8_HUMAN;sp P20042	8	0	7650700	2246000	11763770	2301800	47381000	6348180
tr B2R7B5 B2R7B5_HUMAN;sp Q07666	8	0	7189989	1125067	15410334	1570240	62263310	6264570
tr Q6IAM0 Q6IAM0_HUMAN;sp O7582	8	0	7072800	1458680	10807000	1980700	11757000	3845500
sp O43852-9 CALU_HUMAN;sp O43852	8	0	5929700	2375810	4756000	1839110	2610800	1829720
sp Q9ULH7-4 MKL2_HUMAN;sp Q9ULH	8	0	5929300	0	1533300	0	9667400	0
sp Q9ULC4 MCTS1_HUMAN;sp Q9ULC	8	0	5784500	3863500	18980857	898770	58754000	1178400
sp P18583-6 SON_HUMAN;sp P18583-	8	0	5595300	79908	1326600	863952	6585600	128190
tr E9PC52 E9PC52_HUMAN;tr Q6FHO0	8	0	4981800	182470	6708700	1510000	0	1048300
sp P06132 DCUP_HUMAN;tr B4DEM5	8	0	4785990	0	738410	3283900	11707200	1681800
tr Q53HJ0 Q53HJ0_HUMAN;sp P61201	8	0	4686600	1386300	12197000	433870	25174000	1747390
sp P25398 RS12_HUMAN	8	0	4558149	12128691	14994013	9825470	220254710	30183340
tr B3KUHO B3KUHO_HUMAN;sp Q8WU	8	0	4171600	489910	9975700	249960	0	0
tr A0A024R3W7 A0A024R3W7_HUMAN	8	0	3844890	2479200	8410600	1937885	66393480	4368160
sp P38606-2 VATA_HUMAN;sp P38606	8	0	3823900	1916900	6355400	1471700	3530500	593760
sp Q5TDH0 DDI2_HUMAN;sp Q5TDH0-	8	0	3641848	1162100	2500984	733330	11598919	2413200
tr F8W1R7 F8W1R7_HUMAN;sp P6066	8	0	3542330	3469400	17024000	3268289	141850000	10982000
sp Q16186 ADRM1_HUMAN;tr B4DMP	8	0	3483441	2112200	12043607	2082800	11340220	12714667
tr A8K6Q8 A8K6Q8_HUMAN;sp P0278	8	0	3298330	0	1899522	476530	180860	132410
sp Q13564-3 ULA1_HUMAN;sp Q13564	8	0	3291800	1240000	2383126	1192400	5076380	1966320
sp P56537 IF6_HUMAN;sp P56537-2 I	8	0	3207020	3010580	4652200	2340920	61249000	5813090
sp P31350 RIR2_HUMAN;tr D6W4Z6 D	8	0	3145100	0	3533800	967470	13340100	748350
sp Q96BY7 ATG2B_HUMAN;tr B4DYO	8	0	3082580	0	792920	0	4056200	0
sp Q9HD26-2 GOPC_HUMAN;sp Q9HD	8	0	2934400	0	2144700	0	896300	0
tr Q8TB01 Q8TB01_HUMAN;tr Q6NWX	8	0	2843480	598014	1698200	1328720	0	58146
tr V9HVZ6 V9HVZ6_HUMAN;sp Q9274	8	0	2808650	0	1385791	299580	17888300	0
sp Q92917 GPKOW_HUMAN	8	0	2787137	311270	6530900	303900	3506300	0
tr Q8IVQ8 Q8IVQ8_HUMAN;tr Q0VG7	8	0	2661300	0	834160	0	5333610	268570
tr A0A024R0H7 A0A024R0H7_HUMAN;	8	0	2580000	452040	6312600	1656400	3832050	3023000
sp Q53G59 SNU2_HUMAN;sp Q53G59	8	0	2404160	1489500	6820300	253100	12022000	927170
tr J3QR09 J3QR09_HUMAN;tr J3KTE4	8	0	2383970	710770	5602000	302107	85403620	1254570
tr Q6FI03 Q6FI03_HUMAN;tr Q5U0Q1	8	0	2278500	538460	4696600	1345600	10441000	2082140
sp Q9BTE3-2 MCMBP_HUMAN;sp Q9B	8	0	2144800	668380	4478300	644490	528610	0
tr A0A024R3R7 A0A024R3R7_HUMAN;t	8	0	2029300	0	1081300	0	818350	0
tr A2RUU9 A2RUU9_HUMAN;tr A6NNI	8	0	1932900	0	898080	0	0	0
tr A8K964 A8K964_HUMAN;sp Q9H307	8	0	1885800	490510	741120	319040	21822000	928240
sp P31153 METK2_HUMAN;tr B4DEX8	8	0	1799400	1029300	3380600	441870	0	976787
sp P30154-4 2AAB_HUMAN;sp P30154	8	1	1578500	4027920	2137715	0	1581914	0
sp Q5TFE4 NT5D1_HUMAN;sp Q5TFE4	8	0	1568100	366800	3253700	0	11999680	974580
sp Q5RKV6 EXOS6_HUMAN	8	0	1510762	1082100	3638661	1227571	20085000	2150500
tr M0QY97 M0QY97_HUMAN;sp Q9UP	8	0	1430300	0	252820	0	14100000	0
tr B7ZKR8 B7ZKR8_HUMAN;sp Q86UV	8	0	1382500	0	0	0	9613600	395260
sp O75116 ROCK2_HUMAN;tr E9PF63	8	0	1349600	91266	234120	0	644230	500720
tr Q53GF5 Q53GF5_HUMAN;tr A0A024	8	0	1234190	0	0	0	516760	1688990
tr B5BU08 B5BU08_HUMAN;sp Q01081	8	0	1231630	1040000	2139096	488940	26667240	1297030
sp Q04726-2 TLE3_HUMAN;sp Q04726	8	0	1195200	280220	2266490	1155270	10686100	963220
sp Q96JB5 CK5P3_HUMAN;sp Q96JB5-	8	0	1174800	0	2430000	185510	6999700	0
tr A0A024R830 A0A024R830_HUMAN;s	8	0	1157033	1457800	4213600	1070800	16879000	2108600
sp Q15269 PWP2_HUMAN;tr C9J544 C	8	0	1154300	0	773660	0	10576600	168550
tr A0A024R7V6 A0A024R7V6_HUMAN;	8	0	1139200	723330	3112200	274980	2195700	546430

sp P49720 PSB3_HUMAN;tr A0A087W	8	0	1111420	214280	0	200700	493050	2050520
tr A0A024R3J7 A0A024R3J7_HUMAN;sp	8	0	1107300	0	1391410	0	0	0
sp Q92793-2 CBP_HUMAN;sp Q92793	8	0	1083900	0	308700	73249	5349900	0
sp Q15723-4 ELF2_HUMAN;tr B7Z720	8	0	1083700	164850	2894100	0	4412700	0
tr B3KNH5 B3KNH5_HUMAN;tr A0A02	8	0	1081800	0	2833400	0	10080000	704630
sp Q99549 MPP8_HUMAN;sp Q99549-	8	0	1051800	0	346880	0	7662440	0
sp O75396 SC22B_HUMAN;tr i1VE16 i	8	0	1051170	632660	2495588	254460	7772000	585310
tr A0A0A0MSK6 A0A0A0MSK6_HUMAN	8	0	1020100	77102.2	981270	30037.7	2000400	35702.4
tr i6L9E8 i6L9E8_HUMAN;sp Q8NCA5-	8	0	1007500	0	2460900	367770	9511400	0
tr Q2TAM5 Q2TAM5_HUMAN;tr E9PKf	8	0	998960	0	2202100	395000	10410000	848900
tr Q5T6L4 Q5T6L4_HUMAN;tr A8KAP9	8	0	960860	686340	1605950	0	2826570	1291000
tr Q53EL3 Q53EL3_HUMAN;tr B5BU52	8	0	914450	262910	1107500	489330	6925200	0
sp Q9UBQ7 GRHPR_HUMAN;tr Q5M72	8	0	901960	1667780	3037800	1164070	45297870	3145380
tr J3KMX2 J3KMX2_HUMAN;sp Q9292	8	0	858820	0	1214000	55368	2637200	0
tr Q53F98 Q53F98_HUMAN;tr B2RBY4	8	0	836900	126250	1629700	0	8064800	224920
tr Q6IT96 Q6IT96_HUMAN;sp Q13547	8	0	815340	167540	1545700	42058	2914500	53500
tr Q5JRC6 Q5JRC6_HUMAN;sp Q8iWS	8	0	807190	80436	1506900	199800	7670900	69644
sp P53582 MAP11_HUMAN;tr Q5CZ91	8	0	805920	568070	3461400	0	6664600	774880
tr HOYN26 HOYN26_HUMAN;tr Q6PKH	8	0	788640	3716627	4663290	1217909	50933000	7118720
sp P55735 SEC13_HUMAN;sp P55735-	8	0	772270	1062400	2417401	317480	36362840	432720
tr A8K6G9 A8K6G9_HUMAN;sp Q9262	8	0	710640	0	639060	0	6511500	0
tr Q149P1 Q149P1_HUMAN;tr Q149P0	8	0	685620	117180	553850	437200	5397200	72979
tr Q53HN4 Q53HN4_HUMAN;sp O002	8	0	668514	194350	2970800	340407	20663500	5677400
tr A0A024R5K8 A0A024R5K8_HUMAN;s	8	0	652970	1014700	3897900	510890	2063300	0
sp Q7Z478 DHX29_HUMAN;tr A0A087	8	0	648880	113190	1118060	0	5567508	107460
tr E7EU96 E7EU96_HUMAN;tr B5BUH5	8	0	644980	0	0	0	4782700	441330
tr G5E9A6 G5E9A6_HUMAN;tr Q6P453	8	0	596590	0	707440	0	5625700	600585
sp Q8NFH5-2 NUP53_HUMAN;tr A8K3	8	0	577210	1349500	2982400	1769111	57815070	8302400
tr A0A024QZM5 A0A024QZM5_HUMAN	8	0	520670	0	544300	0	48940000	39764
sp Q8N201 INT1_HUMAN;tr A4D212 A	8	0	479370	0	590500	0	4978820	0
sp P62316 SMD2_HUMAN;sp P62316-2	8	0	468520	805150	1579300	423630	27033000	4183700
tr Q5U0A0 Q5U0A0_HUMAN;sp P2806	8	0	453923	1238640	610989	1061110	11924110	4359720
sp Q5JPE7-2 NOMO2_HUMAN;sp Q15	8	0	429190	79389	162410	0	321130	0
sp Q9UHB7 AFF4_HUMAN;sp Q9UHB7	8	0	391410	0	0	0	6303100	0
tr A0A024R496 A0A024R496_HUMAN;s	8	0	369070	151440	875860	87402	2374800	0
tr A0A024R1I3 A0A024R1I3_HUMAN;sp	8	0	346320	202250	821070	233710	12622000	525740
tr A8K1E1 A8K1E1_HUMAN;sp P20585	8	0	322420	0	667270	0	5514000	0
tr B7Z7P2 B7Z7P2_HUMAN;tr Q59EA0	8	0.003	309230	0	0	0	0	0
tr Q5U071 Q5U071_HUMAN;sp P2658	8	0	283845	371196	131330	156766	550550	2554191
tr A0A0A0MSW4 A0A0A0MSW4_HUM	8	0	249110	270290	355060	0	6041420	0
sp P53004 BIEA_HUMAN;tr C9J1E1 C9	8	0	246680	416880	966530	129370	6698900	0
sp Q5T1M5 FKB15_HUMAN;tr A0A0A0	8	0	212240	111250	0	0	4075200	89481
sp Q96H79 ZCCHL_HUMAN;sp Q96H79	8	0	201130	209770	1069220	1134050	12980000	229230
tr B4DZQ5 B4DZQ5_HUMAN;tr A0A02	8	0	192080	0	0	0	8037200	0
tr Q53G61 Q53G61_HUMAN;tr Q53G21	8	0	175280	0	566560	0	9851800	1398100
sp Q9BTA9-2 WAC_HUMAN;sp Q9BTA	8	0	126520	517750	2701400	653890	5505500	660670
sp Q86V81 THOC4_HUMAN;tr E9PB61	8	0	124750	0	1687400	0	37541000	725630
tr Q86S27 Q86S27_HUMAN;tr Q6FHK9	8	0	112080	711630	976400	0	729170	101590
tr A8K9K8 A8K9K8_HUMAN;sp Q9UNC	8	0	75843	64586	799879.7	0	14297000	0
sp P08579 RUB2_HUMAN;tr B5BTZ8 B	8	0	74457	0	231400	0	4780270	0
tr A0A024R333 A0A024R333_HUMAN;s	8	0	59259	43752	531700	0	16331000	253390
sp O75155-2 CAND2_HUMAN;sp O751	8	0	0	0	0	0	5924940	0
sp O95834 EMAL2_HUMAN;tr K7EIK7	8	0	0	0	0	0	1597200	0
sp P56270-2 MAZ_HUMAN;sp P56270-	8	0	0	0	0	0	4482450	0
sp Q6P158 DHX57_HUMAN;sp Q6P158	8	0	0	0	0	0	3485400	0
sp Q6S8J3 POTEE_HUMAN;sp A5A3E0	8	1	0	0	2251600	0	4109800	0
sp Q96CN7 ISOC1_HUMAN;tr D6RGE2	8	0	0	0	0	0	402680	0
tr A0A024R1Z6 A0A024R1Z6_HUMAN;s	8	0	0	0	727870	0	0	0
tr B2R7G4 B2R7G4_HUMAN;sp P32780	8	0	0	0	0	0	7036400	0
tr B2R9I9 B2R9I9_HUMAN;tr A0MNP2	8	0	0	0	0	0	18434600	0
tr B4DG22 B4DG22_HUMAN;tr B7ZB17	8	0	0	0	0	0	1764700	0
tr B4DWB1 B4DWB1_HUMAN;sp O606	8	0	0	0	1622400	0	4768600	0
tr S4R3H4 S4R3H4_HUMAN;tr Q69YJ6	8	0	0	0	0	0	5210200	58792
sp Q9Y315 DEOC_HUMAN;tr G3V158	8	0	0	0	386950	0	6176900	232540
tr Q9NX34 Q9NX34_HUMAN	8	0	0	0	0	0	2110700	583160
tr Q5T7C4 Q5T7C4_HUMAN;tr Q5T7C6	8	0	0	0	0	0	418090	1289110
sp P22059 OSBP1_HUMAN;tr B4DR25	8	0	0	0	0	0	0	188310
tr A8K067 A8K067_HUMAN;tr B4DI19	8	0	0	0	558170	0	0	356446
tr A8KAQ5 A8KAQ5_HUMAN;tr A0A02	8	0	0	0	0	0	0	29021
tr B4E2H2 B4E2H2_HUMAN;tr B4DZD5	8	1	0	0	0	0	0	514110
tr J3KN67 J3KN67_HUMAN;sp P06753	8	0.006803	0	0	0	0	0	251980
tr Q6NVY0 Q6NVY0_HUMAN;tr A0A02	8	0	0	0	0	0	0	1018200
tr Q5W009 Q5W009_HUMAN;sp Q96I2	8	0	0	0	967100	99786	10325000	204160
sp Q9NPI6-2 DCP1A_HUMAN;tr Q3LIB	8	0	0	0	1173800	129280	9420100	0
sp Q8N335 GPD1L_HUMAN;tr C9JIM46	8	0	0	408170	933820	166590	9860600	0

tr Q96CV8 Q96CV8_HUMAN;sp P5288	8	0	0	367820	1299800	387580	1342700	1500000
sp Q8N7H5-3 PAF1_HUMAN;tr A0A02	8	0	0	0	730350	236500	10749000	624200
tr V9HW41 V9HW41_HUMAN;sp P610	8	0	0	308330	99889	55365	1770700	5270700
tr B2R514 B2R514_HUMAN;tr E7EM64	8	0	0	0	1800600	1038500	14573000	1501990
sp P17174 AATC_HUMAN;tr B7Z112 B	8	0	0	0	0	276865	0	367240
tr Q5RKT7 Q5RKT7_HUMAN;tr B2RDW	7	0	99724000	48617000	85700000	41241700	230828000	134368000
sp P47929 LEG7_HUMAN	7	0	47520060	516540	1903750	2015990	11467750	2219680
sp P46783 RS10_HUMAN;tr Q59GE4 C	7	0	31117680	6201390	31002950	3285690	79371090	9470910
sp P30050 RL12_HUMAN;tr Q59F19 Q	7	0	19995400	27390580	49191870	19613420	293008940	33797170
tr Q53F20 Q53F20_HUMAN;sp Q98TTC	7	0	17339000	4480900	22754000	5736527	185540000	26117983
sp Q13867 BLMH_HUMAN;tr K7ESE8 H	7	0	15333330	1205420	1740580	2508710	1901270	1638360
sp P62277 RS13_HUMAN;tr J3KMX5 J	7	0	12146840	6116870	17016860	6499480	191287420	23802460
tr ESR199 ESR199_HUMAN;tr A0A024R9	7	0	11878200	33551500	24733070	23979210	213130000	46806000
tr Q9BTQ7 Q9BTQ7_HUMAN;tr A0A02	7	0	11734770	5812340	28355490	2982068	192509070	11893065
tr A0A024R4E2 A0A024R4E2_HUMAN;s	7	0	10476300	3856941	21995780	3260400	38419370	6234654
sp P31151 S10A7_HUMAN;sp Q865G5	7	0	9452320	2136620	2276000	3443180	2662520	3371400
tr Q6MZWO Q6MZWO_HUMAN;sp P01	7	0	9381580	2663640	2752000	4605610	6905550	5388900
tr A0A090N7V5 A0A090N7V5_HUMAN	7	0	7857130	2168660	1585910	967390	637510	1310940
sp P61026 RAB10_HUMAN;tr Q53T70	7	0	7634270	190390	5678600	91529	3007090	216540
tr B7Z1N6 B7Z1N6_HUMAN;tr B7Z1Y2	7	0	6647600	0	20529000	0	12453870	1069878
tr Q6FGX3 Q6FGX3_HUMAN;tr A0A02	7	0	5758010	4839382	11422110	2367300	17651000	2592600
tr A0A024QZY1 A0A024QZY1_HUMAN;	7	0	5153700	2189800	9480200	1609100	42779000	4023400
tr A8K517 A8K517_HUMAN;sp P62266	7	0	5122500	3508300	5656570	2229220	127187150	4270870
sp Q8IXH7-4 NELFD_HUMAN;sp Q8IXH	7	0	4498516	754020	5899200	819832	8507100	438240
sp Q9UN52 CSN3_HUMAN;sp Q9UN52	7	0	4452360	397830	10221000	846470	15725000	2601288
sp P13807-2 GYS1_HUMAN;tr Q53ER0	7	0	4359710	0	1935000	0	8547510	629760
sp A8K2U0 A2ML1_HUMAN;tr Q6ZWK	7	0	4329490	0	0	0	0	0
sp Q9BV20 MTNA_HUMAN;sp Q9BV20	7	0	4107100	0	4536014	966049	13576610	2646567
tr Q5U0E4 Q5U0E4_HUMAN;tr B6E4X6	7	0	3373300	864930	6539900	1322400	7336700	811430
tr Q6PKC2 Q6PKC2_HUMAN;tr Q86Y74	7	0	3369500	1976901	8280500	2319100	20541000	4621066
tr Q5H909 Q5H909_HUMAN;sp Q9UNF	7	0	3365700	950780	2915900	937020	7472800	434820
tr K7EJR3 K7EJR3_HUMAN;tr Q5U0B3	7	0	2881301	2501100	13966000	1468900	64546000	2748900
tr B4DEN6 B4DEN6_HUMAN;tr A0A02	7	0	2821200	0	10015000	338020	6164000	0
sp Q5JRA6-2 MIA3_HUMAN;sp Q5JRA	7	0	2723800	330900	2395530	0	0	0
tr B4DLT1 B4DLT1_HUMAN;sp Q75436	7	0	2575395	0	360550	421740	11308510	1607250
tr V9HW95 V9HW95_HUMAN;sp Q152	7	0	2565700	238510	3772433	909670	1963300	1343140
sp Q5VUA4 ZN318_HUMAN;sp Q5VUA	7	0	2490120	0	219300	0	1664400	0
sp Q9H3N1 TMX1_HUMAN;tr B4DZX7	7	0	2462600	2008300	3803700	542800	1156800	299450
sp Q5JVF3-3 PCID2_HUMAN;tr A8K09	7	0	2417900	0	2487700	369050	9120500	510920
tr B3KU09 B3KU09_HUMAN;sp Q5XPI4	7	0	2331000	0	1782600	412510	2179900	212140
sp O00139-2 KIF2A_HUMAN;tr B0AZ5	7	0	2109070	387360	3616480	219110	5472700	494620
sp O60885 BRD4_HUMAN;sp O60885-2	7	0	2004300	387970	1268500	384940	13386000	783770
sp Q9UPN7 PP6R1_HUMAN;tr B4DLT9	7	0	1867500	311670	1190700	73710	5547500	412900
tr A0A024R411 A0A024R411_HUMAN;s	7	0	1821000	225470	2482140	478910	12934150	0
sp Q6UN15-3 FIP1_HUMAN;sp Q6UN1	7	0	1744700	216550	2669600	384410	10359000	714340
sp Q5VTR2 BRE1A_HUMAN;tr Q05DCC	7	0	1683000	0	500970	0	4025800	0
tr B7Z341 B7Z341_HUMAN;tr F8WBF9	7	0	1661500	397847	4321780	433765	10535090	487550
tr B2RCD8 B2RCD8_HUMAN;sp Q8IU8	7	0	1629200	1138200	4351200	1488200	4943700	0
tr Q6IPN0 Q6IPN0_HUMAN;tr F8W914	7	0	1623210	565422	2307100	581768	413410	477026
tr D3DXC9 D3DXC9_HUMAN;sp P3489	7	0	1580294	330010	2835900	268860	7828600	779560
sp Q9UBW8 CSN7A_HUMAN;tr Q567U	7	0	1532320	936510	3482900	0	32195000	1762500
tr A1L3Z9 A1L3Z9_HUMAN;sp Q2TAL8	7	0	1462180	323010	646790	369560	9786435	238210
sp Q9H054-2 DDX47_HUMAN;tr Q53G	7	0	1423200	169930	2446600	337160	6959200	0
sp Q14694 UBP10_HUMAN;sp Q14694	7	0	1419200	0	319000	0	0	209040
tr B2RAH5 B2RAH5_HUMAN;sp O1497	7	0	1393539	0	1036300	0	7470600	253510
sp Q96RN5-3 MED15_HUMAN;tr G3V1	7	0	1382000	608730	2480000	695995	6163455	1204500
sp Q7L1Q6-2 BZW1_HUMAN;tr A0A02	7	0	1356400	572510	2811900	624860	15866330	722100
tr B5BU53 B5BU53_HUMAN;tr B2R9L6	7	0	1348300	141310	2140200	345580	4544500	535430
tr Q59GQ7 Q59GQ7_HUMAN;tr A8K5B	7	0	1348200	132030	690140	164580	6749900	0
sp Q9NQW7-4 XPP1_HUMAN;sp Q9N	7	0	1341715	328840	574560	259840	1166800	472840
tr Q6I9S2 Q6I9S2_HUMAN;sp Q13573	7	0	1336800	437210	1674200	733550	4972000	201890
sp Q43237-2 DC1L2_HUMAN;tr A0A02	7	0	1292400	765140	7620300	860080	7019780	3011400
tr E9PB90 E9PB90_HUMAN;tr A8K2U2	7	0	1251100	0	550540	0	1684700	0
tr HOY2Y8 HOY2Y8_HUMAN;sp Q15942	7	0	1214500	386803	5456300	604660	13956000	582440
tr E9PQP3 E9PQP3_HUMAN;tr G5E9L0	7	0	1212600	1195568	9642159	1460300	17255650	2317942
tr A8K6X9 A8K6X9_HUMAN;sp Q6PD6	7	0	1195000	0	422640	0	10367000	187200
tr A0A024RAC6 A0A024RAC6_HUMAN;	7	0	1179100	66003	306600	0	4740000	369950
sp Q96SI9-2 STRBP_HUMAN;tr V9HW	7	0.000357	1137790	0	0	0	6023900	0
sp Q6XQNG-3 PNCB_HUMAN;sp Q6XC	7	0	1123500	0	3040800	0	8839300	1151800
sp Q7Z4H3-2 HDDC2_HUMAN;sp Q7Z4	7	0	1066400	516390	2008900	612130	15453000	1948400
tr F8W7C6 F8W7C6_HUMAN;tr A0A08	7	0	1032092	407070	4657620	0	29375000	2550600
sp Q92572 AP3S1_HUMAN;tr B2R4I8	7	0	1015600	3901022	6049840	1239700	16691000	255140
sp Q7Z7L1 SLN11_HUMAN;tr K7ER38	7	0	1009700	0	916170	0	6029500	0
sp Q9NX46 ARHL2_HUMAN;tr B4DHV5	7	0	964760	0	2186500	0	947450	1076249
tr V9HWA6 V9HWA6_HUMAN;sp P60	7	0	915110	665940	3629700	461140	9024500	1040767

tr Q8IW76 Q8IW76_HUMAN;tr B7ZKK7	7	0	861110	0	2985100	0	11303000	0
sp P14324-2 FPP5_HUMAN;sp P14324	7	0	859400	1237300	1633100	0	17157000	4065730
tr Q53GF0 Q53GF0_HUMAN;sp Q8NFV	7	0	854080	0	2601500	111820	10213540	544850
tr A0PJ47 A0PJ47_HUMAN;sp Q14151	7	0.00036	845750	0	0	0	0	0
sp Q9BS26 ERP44_HUMAN	7	0	844580	308690	1375910	0	9480800	0
sp Q9Y266 NUDC_HUMAN;tr Q9H2R7	7	0	837350	1260180	1771000	310220	2077900	1864106
sp O95758-1 PTBP3_HUMAN;sp O9575	7	0	820750	0	3172200	0	8544500	0
tr Q6FH36 Q6FH36_HUMAN;sp O4344	7	0	820670	935140	1695880	716300	31196000	1954248
tr V9HW40 V9HW40_HUMAN;sp O758	7	0	797300	0	1647600	46899	14645000	1393200
tr B2R8A2 B2R8A2_HUMAN;tr A0A024	7	0	770820	0	1221060	0	11909000	1676290
sp Q96L92-3 SNX27_HUMAN;sp Q96L9	7	0	767310	0	832760	0	7341000	216820
sp P48449-3 ERG7_HUMAN;sp P48449	7	0	763490	378240	1251600	0	339390	0
sp Q12802-4 AKP13_HUMAN;sp Q1280	7	0	738400	0	250280	228710	13350300	112720
tr Q9UHT7 Q9UHT7_HUMAN	7	0.00682	737830	0	0	0	0	0
tr Q05BW9 Q05BW9_HUMAN;tr Q6IA	7	0	718760	0	3146200	0	2688040	0
tr Q53F37 Q53F37_HUMAN;sp Q9Y6B6	7	0	703210	0	8058600	0	8984480	1488100
sp Q55W79 CE170_HUMAN;tr A6H8X9	7	0	630880	0	631660	0	4402700	251120
tr B3KR89 B3KR89_HUMAN;sp O95785	7	0	629681	130000	433100	0	3324800	90226
sp P46939 UTRO_HUMAN;sp P46939-2	7	0	622250	0	444840	0	1805940	0
tr V9HW74 V9HW74_HUMAN;sp P099	7	0	621390	484190	1995137	200530	8972800	2133900
sp P22307-7 NLTP_HUMAN;sp P22307	7	0	517048	2071500	4349140	643130	9003070	1536400
tr Q53R19 Q53R19_HUMAN;sp O15144	7	0	484260	298090	1550900	720290	2337800	1103000
tr B2RAJ6 B2RAJ6_HUMAN;tr A0JP11	7	0	473610	0	686980	0	9027500	355152
tr B7ZAC0 B7ZAC0_HUMAN;tr B4DK06	7	0	440970	0	326830	0	4811800	437150
tr Q5TDE9 Q5TDE9_HUMAN;sp Q9BSD	7	0	440680	0	1395100	0	18392000	1436900
sp Q92995-2 UBP13_HUMAN;sp Q9299	7	0	440550	0	320480	0	2438200	204830
sp Q9C0J8 WDR33_HUMAN;tr C9J8B4	7	0	424630	0	173470	0	4721700	0
tr B2R7E8 B2R7E8_HUMAN;sp P15927	7	0	418991	437090	1825400	251610	10950000	1127900
sp Q9NPQ8-4 RIC8A_HUMAN;sp Q9NI	7	0	401360	0	398570	268719	1761400	0
tr E5KT65 E5KT65_HUMAN;sp P19388	7	0	399930	0	797879	19671	18900000	711880
tr A8MZF9 A8MZF9_HUMAN;sp P5503	7	0	394010	0	3793600	0	5172400	234430
sp Q86VM9 ZCH18_HUMAN;tr E7ERS3	7	0	392530	2729115	0	1032190	6136400	374940
tr Q549N5 Q549N5_HUMAN;sp Q9Y5N	7	0	374970	508160	1237800	200120	648680	0
tr V9HWJ8 V9HWJ8_HUMAN;sp P612E	7	0	361720	99505	3156900	235860	12411890	1112300
tr B2RDN3 B2RDN3_HUMAN;sp Q9Y5Y	7	0	319360	502360	1539600	483030	14790000	966910
sp Q08379 GOGA2_HUMAN;sp Q08379	7	0	318870	0	238740	0	4956600	0
tr Q53SW3 Q53SW3_HUMAN;sp Q9BP	7	0	297170	372610	8356100	265630	20815000	0
tr A0A024R4U0 A0A024R4U0_HUMAN;	7	0	276110	0	911800	319840	6426100	685100
sp P25490 TYY1_HUMAN;tr H0YJV7 H	7	0	268340	153320	2320200	153300	8932175	627450
tr D3YTB1 D3YTB1_HUMAN;tr A0A024	7	0	246180	0	1929570	52987	33288000	1861120
tr Q6FHF7 Q6FHF7_HUMAN;sp Q9269	7	0	234610	0	1965900	0	2540270	0
sp Q9BZV1-2 UBXN6_HUMAN;sp Q9BZ	7	0	231050	0	1978900	0	6885800	0
tr A0A024RB09 A0A024RB09_HUMAN;	7	0	221560	313430	56285	420760	0	0
sp P55795 HNRH2_HUMAN;tr B4DFK9	7	0	218440	0	548320	0	968150	0
sp Q9NPF4 OSGEP_HUMAN;tr G3V4G	7	0	217100	858050	1162300	0	12812000	1209500
sp Q9H0U4 RAB18_HUMAN;tr E9PLD0	7	0	212680	332340	876820	188870	933990	332220
tr Q8TC07-2 TBC15_HUMAN;sp Q8TC	7	0	171750	0	971200	0	4721400	0
tr C9JFR7 C9JFR7_HUMAN;tr G4XXL9	7	0	149800	107850	0	57785	372240	3469300
tr Q5VXV3 Q5VXV3_HUMAN;sp Q0110	7	0	149010	1304590	1393330	821000	871480	5315490
tr Q9H2G0 Q9H2G0_HUMAN;tr Q5W7	7	0	136710	0	958390	0	3306200	0
sp Q15018 F175B_HUMAN	7	0	128150	0	613160	0	4643700	81704
sp P53384-2 NUBP1_HUMAN;sp P5338	7	0	122400	0	1337400	0	10813210	980690
tr Q6PKD2 Q6PKD2_HUMAN;sp P0DM	7	0.003002	104720	0	0	0	0	0
sp Q14019 COTL1_HUMAN;tr H3BT58	7	0	76352	1105700	614040	1937000	6499600	754500
sp Q9BUH6 CI142_HUMAN;sp Q9BUH6	7	0	76070	0	133640	0	4500200	463990
tr F5GXF5 F5GXF5_HUMAN;sp Q12830	7	0	52883	13211	0	0	4706100	40085
sp P14635-2 CCNB1_HUMAN;tr E9PC9	7	0	0	0	1049800	0	1293800	0
sp P36507 MP2K2_HUMAN;tr G5E9C7	7	0	0	0	973660	0	4818530	0
sp P54619-2 AAKG1_HUMAN;tr A0A02	7	0	0	0	468500	0	4021120	0
sp Q01804 OTUD4_HUMAN;sp Q01804	7	0	0	0	172600	0	4021600	0
sp Q13330-3 MTA1_HUMAN;tr E7ESY4	7	0	0	0	489510	0	1144600	0
sp Q5MIZ7-3 P4R3B_HUMAN;sp Q5MI	7	0.009394	0	0	0	0	816640	0
sp Q9NTX5-6 ECHD1_HUMAN;sp Q9N	7	0	0	0	480710	0	4090000	0
sp Q9UBD5-3 ORC3_HUMAN;tr B4E014	7	0	0	0	0	0	4926200	0
sp Q9Y2X3 NOP58_HUMAN;tr B3KN82	7	0	0	0	1016000	0	9622500	0
tr A0A024RCZ8 A0A024RCZ8_HUMAN;	7	0	0	0	0	0	2928170	0
tr F8W950 F8W950_HUMAN;tr A8MU5	7	1	0	0	0	0	930010	0
tr Q1W6G4 Q1W6G4_HUMAN;sp Q9N	7	0.006548	0	0	0	0	733060	0
tr Q53FR9 Q53FR9_HUMAN;sp Q9P00	7	0	0	0	112030	0	5486500	0
tr V9HWG0 V9HWG0_HUMAN;sp P455	7	0	0	0	1889600	0	3337600	86830
tr X1WI28 X1WI28_HUMAN;tr X5D2T3	7	0	0	0	3266200	0	9784700	479590
sp O95983-2 MBD3_HUMAN;sp O9598	7	0	0	0	0	0	5177800	269850
tr A0A023T6R1 A0A023T6R1_HUMAN;s	7	0	0	0	0	0	5620260	1074100
tr V9HW92 V9HW92_HUMAN;sp O001	7	0	0	0	392900	0	3545060	1270900
sp P33316-2 DUT_HUMAN;tr H0YNW5	7	0	0	0	0	0	0	1314767



tr A0A024R2U9 A0A024R2U9_HUMAN;	7	0	0	0	0	0	0	502160
tr A0A024RDB0 A0A024RDB0_HUMAN;	7	0	0	0	0	0	0	249630
tr B4DEA3 B4DEA3_HUMAN;tr Q53F10	7	0	0	0	0	0	0	243230
tr D6RGI3 D6RGI3_HUMAN;sp Q9NVA	7	0	0	0	0	0	0	303120
sp Q86U44 MTA70_HUMAN;tr B4E2F6	7	0	0	262960	3219000	0	8711880	0
sp Q8TDD1 DDX54_HUMAN;sp Q8TDD	7	0	0	97676	0	0	8213600	0
tr Q96BS4 Q96BS4_HUMAN;sp P22087	7	0	0	268440	402550	0	8318700	0
tr Q6FHU3 Q6FHU3_HUMAN;sp Q0632	7	0	0	87745	137320	0	750200	506380
sp Q9NRN7 ADPPT_HUMAN;sp Q9NR	7	0	0	60615	30054	0	41704	353780
sp A6NDG6 PGP_HUMAN;tr H3BV17 H	7	0	0	137230	817860	90115	7509000	0
tr B4DRN6 B4DRN6_HUMAN;tr A0A02	7	0	0	434990	3017700	357210	17406000	711240
tr B7Z4Q0 B7Z4Q0_HUMAN;tr H0Y6A0	7	0.001036	0	0	681900	86276	765220	0
tr A0A024R471 A0A024R471_HUMAN;s	7	0	0	223180	951210	322800	15021000	710040
sp Q9BTT0-3 AN32E_HUMAN	7	0	0	248630	544670	321390	2465000	0
tr J3QLE5 J3QLE5_HUMAN;tr Q66K91	7	0	0	216455	186120	204747	8234000	870880
tr F8VR84 F8VR84_HUMAN;tr Q8WYI2	7	0	0	0	0	305021	0	274380
sp P14859-4 PO2F1_HUMAN;sp P1485	7	0	0	397030	0	180090	14724300	2536600
tr Q53FT8 Q53FT8_HUMAN;sp P20618	7	0	0	632625	0	67090	486460	962220
sp P81605 DCD_HUMAN	6	0	161153000	142438000	117114000	150502000	117449000	178818000
tr Q53FU3 Q53FU3_HUMAN;tr A0A024	6	0	19683000	13877000	76857000	7440378	124500000	6126500
sp P01040 CYTA_HUMAN;tr C9J0E4 C9	6	0	13585300	9799200	10506500	10817900	12214900	11597000
tr V9HW34 V9HW34_HUMAN	6	0	9804900	4941800	2847370	5775000	7193670	6676720
tr A0A024RCA7 A0A024RCA7_HUMAN;	6	0	9058040	11053020	33744220	10505100	136780000	41028020
tr Q6NZ52 Q6NZ52_HUMAN;sp P4677	6	0	8457690	3867850	13015800	2554290	140372000	13995140
sp P62847-2 RS24_HUMAN;tr E7ETK0	6	0	7528200	790720	12129000	255280	41975960	2789660
tr V9HWH9 V9HWH9_HUMAN;sp P315	6	0	7468320	3057800	541320	0	194790	4681360
tr Q7RTM4 Q7RTM4_HUMAN	6	0	7016070	2773573	11311975	2519400	33974450	6455190
tr E5RGR0 E5RGR0_HUMAN;sp O75608	6	0	6749110	2804200	12407000	1795807	53327000	2586200
tr B4DUS0 B4DUS0_HUMAN;sp Q9P28	6	0	5961800	353580	9456700	1065600	14247000	1366700
tr B0AZN7 B0AZN7_HUMAN;tr A8K5N	6	0	5898750	679270	683110	2047830	225180	0
sp P42357 HUTH_HUMAN;sp P42357-2	6	0	5494020	0	0	167170	0	0
sp Q58FF6 H90B4_HUMAN	6	0	5280600	3356413	9172834	3030435	9170880	4943190
tr Q59GU5 Q59GU5_HUMAN;sp Q134C	6	0	4489300	2301921	9263630	2024784	14974980	3482500
tr A8K7N0 A8K7N0_HUMAN	6	0	4480250	3804275	10204540	1969600	146085704	6968020
sp P40616-2 ARL1_HUMAN;sp P40616	6	0	4427700	2396000	8477200	1497500	30745100	12069241
tr B2R4S9 B2R4S9_HUMAN;tr A8K9J7	6	0	4378910	3793396	6152370	248483	28749100	2181050
tr Q8N355 Q8N355_HUMAN;tr Q6GMV	6	0	4292500	1996000	3116060	1113490	4573850	2698240
tr K7EQ02 K7EQ02_HUMAN;sp Q96EP5	6	0	4149442	1082000	13779666	1184600	29123000	5677563
tr B7Z5J4 B7Z5J4_HUMAN;sp Q9UI42-2	6	0	4057045	65017	0	0	50154	0
tr B4DHC5 B4DHC5_HUMAN;tr A0A024	6	0	4037200	743250	10640320	779990	12726460	1054300
tr C9JGI3 C9JGI3_HUMAN;tr E5KRG5 E	6	0	4000400	0	0	0	0	0
sp Q02241-2 KIF23_HUMAN;tr H7BYN4	6	0	3793000	168350	1463500	0	2673300	0
sp Q9P035 HACD3_HUMAN;tr H3BS72	6	0	3739340	322140	1583080	633940	2087590	0
sp P61224-2 RAP1B_HUMAN;sp P6122	6	0	3560900	1076182	14010000	5170625	5882800	2873400
sp Q8WYA6-4 CTBL1_HUMAN;sp Q8W	6	0	3450100	624750	6066300	245500	14072000	0
tr A0A024R8P8 A0A024R8P8_HUMAN;s	6	0	3202370	4069100	8658500	2469666	80123460	17875000
sp Q05048 CSTF1_HUMAN;tr B4DDG3	6	0	3173300	158690	2393800	399340	13742000	1492600
sp Q14257 RCN2_HUMAN;sp Q14257-2	6	0	3126900	0	5010600	218340	7265500	1424700
sp Q9H6R4-4 NOL6_HUMAN;sp Q9H6F	6	0	2898900	274790	787850	0	4482500	423880
tr Q5JR08 Q5JR08_HUMAN;tr A0A024F	6	0	2875300	2835800	6984800	2068320	24472000	7552100
sp P49903 SPS1_HUMAN;sp P49903-2	6	0	2678800	2105900	6988300	862758	11642000	1512100
tr B7Z7Z8 B7Z7Z8_HUMAN;tr B7Z8G2	6	0	2668700	568530	4311800	623190	13356000	1755100
tr E9PG22 E9PG22_HUMAN;sp Q8IW35	6	0	2640800	107210	781160	148660	1591500	109840
sp P59998 ARPC4_HUMAN;tr F8WCF6	6	0	2588720	2600600	3721400	1249407	6229100	3941300
tr B4DXG8 B4DXG8_HUMAN;tr D6RIY6	6	0	2565000	198290	5860900	315850	9189560	0
sp Q6PJT7-10 ZC3HE_HUMAN;sp Q6PJ	6	0	2528000	228153	1223870	280290	6011800	715520
sp Q2TAZ0 ATG2A_HUMAN;sp Q2TAZ	6	0	2475200	0	570060	0	4176000	0
tr Q6PKI6 Q6PKI6_HUMAN;tr Q05D43	6	0	2440931	600406	3482600	425640	8967000	1520150
tr A0A024R8M4 A0A024R8M4_HUMAN	6	0	2419100	0	1900600	644280	8587490	1031800
sp P31942-3 HNRH3_HUMAN;sp P3194	6	0	2381640	621300	5055500	355770	14915000	2568080
sp O95155-2 UBE4B_HUMAN;sp O9515	6	0	2280000	0	0	0	4858680	0
sp Q8TCG1 CIP2A_HUMAN;tr A0A087F	6	0	2088920	0	190890	0	1191800	0
sp Q8WWH5 TRUB1_HUMAN;tr B4DZ5	6	0	1989120	0	1479130	132680	1719700	529090
sp Q5UIP0-2 RIF1_HUMAN;sp Q5UIP0	6	0	1886300	0	285710	0	1504940	0
sp P12532 KCRU_HUMAN;sp P12532-2	6	0	1883200	0	3123100	0	4849000	2321300
tr B0AZM4 B0AZM4_HUMAN;tr A8K6F	6	0	1865500	97746	663840	0	3357300	50897
tr Q71UA6 Q71UA6_HUMAN;sp Q1575	6	0	1829000	0	1993200	0	0	0
sp O00487 PSDE_HUMAN;tr Q53TH1 C	6	0	1758620	1981866	5947100	1483505	27175000	5906939
tr E9PRQ7 E9PRQ7_HUMAN;tr A0A024	6	0	1690679	269330	0	1225070	13072400	2263700
tr E9PIN3 E9PIN3_HUMAN;sp Q9UBU5	6	0	1684430	222030	2048310	0	5443530	0
tr A0A087WXM6 A0A087WXM6_HUMA	6	0	1664890	2612100	7410288	0	96602040	5901210
tr B4E054 B4E054_HUMAN;tr A0A024F	6	0	1494400	113860	2874700	121560	1776600	157620
tr B2R7C2 B2R7C2_HUMAN;sp Q43264	6	0	1490700	328200	3397200	107720	4501600	0
sp Q9UFC0 LRWD1_HUMAN;tr H7C556	6	0	1484040	0	2328300	0	8859300	0
sp Q96G03 PGM2_HUMAN;tr B4DN40	6	0	1418500	143567000	4270000	143932000	19448900	197262000

sp Q9BW27-3 NUP85_HUMAN;sp Q9B	6	0	1402200	0	2930400	498580	10741851	0
tr Q68CN2 Q68CN2_HUMAN;tr Q715V	6	0	1347800	0	0	0	4247100	167030
sp Q68CP9-3 ARID2_HUMAN;sp Q68C	6	0	1328400	0	926410	229120	4301100	0
tr A0A024R388 A0A024R388_HUMAN;s	6	0	1262800	426560	3173500	421610	8787200	1044300
sp O15400-2 STX7_HUMAN;sp O15400	6	0	1204470	175880	2206460	0	5136300	0
tr B7Z9T5 B7Z9T5_HUMAN;tr A0A024R	6	0	1195100	1375000	2753800	251510	4460300	0
sp Q9C0C9 UBE2O_HUMAN;tr K7ES11	6	0	1176100	0	0	0	1912900	0
sp Q15056-2 IF4H_HUMAN;sp Q15056	6	0	1146920	1330500	3195100	1077100	31913180	1575400
sp O75376-2 NCOR1_HUMAN;tr A0A0	6	0	1128200	96204	374370	187910	5354800	0
tr Q8N959 Q8N959_HUMAN;tr B3KU6	6	0	1068000	1601600	3369800	908910	10487559	267410
sp P13861-2 KAP2_HUMAN;tr A8KAH7	6	0	1053900	422180	3354300	0	4626000	532170
tr E5RHW4 E5RHW4_HUMAN;sp O949	6	0	994810	0	2607200	0	0	0
sp P20339-2 RAB5A_HUMAN;tr A0A02	6	0	969910	982220	1595700	680960	994420	705040
tr F8VSC5 F8VSC5_HUMAN;sp Q6P3W	6	0	944310	181300	338690	0	3128500	537860
sp Q92783-2 STAM1_HUMAN;tr B2RA	6	0	926160	0	2051500	0	6094600	0
sp Q14574-2 DSC3_HUMAN;tr A8K6T3	6	0	900810	3052660	1153020	4786830	4082050	1177910
sp Q8IZ83-3 A16A1_HUMAN;sp Q8IZ8	6	0	888610	0	1866200	0	6355600	141210
tr Q0UJ56 Q0UJ56_HUMAN;tr B4E0U6 E	6	0	878100	772030	800650	360310	3024200	2579508
sp Q9NZL4 HPBP1_HUMAN;sp Q9NZL4	6	0	872590	0	2329900	199840	9521000	0
sp P06730 IF4E_HUMAN;sp P06730-2	6	0	814059	818790	2357770	594090	32192656	1043800
sp Q14444-2 CAPR1_HUMAN;sp Q144	6	0	770350	0	1263140	0	4431880	1283900
sp Q01433-5 AMPD2_HUMAN;sp Q014	6	0	769920	133370	516310	0	4325900	1688800
tr E9PID8 E9PID8_HUMAN;tr B4DUD5	6	0	761010	400340	1395000	489480	4909500	0
sp Q96P48-3 ARAP1_HUMAN;sp Q96P	6	0	675960	0	330470	0	1891200	195060
tr H7BZJ3 H7BZJ3_HUMAN	6	0	665730	511630	707290	358610	892260	440070
tr A8K9U0 A8K9U0_HUMAN;sp Q9BWI	6	0	655800	0	1406100	251760	4153960	391420
sp Q16629-3 SRSF7_HUMAN;sp Q1662	6	0	644360	674800	876430	478340	6642700	4604220
sp Q43719 HTSF1_HUMAN;tr B4DRS4	6	0	629930	0	132050	0	5961200	54779
sp Q9UJU6 DBNL_HUMAN;sp Q9UJU6-	6	0	600350	127130	1025000	0	6117300	274750
sp P50748 KNTC1_HUMAN;sp P50748-	6	0	600330	0	519620	117950	3418200	0
tr B4DYG5 B4DYG5_HUMAN;sp P42356	6	0	572030	0	467900	0	3179000	0
tr A0A024R9B5 A0A024R9B5_HUMAN;s	6	0	558820	282360	3324400	0	9327100	162830
tr Q5U025 Q5U025_HUMAN;sp P62330	6	0	558430	4134500	1393300	997060	10928000	2236300
sp O95433 AHS1_HUMAN;tr H0YJG7	6	0	552950	0	1596200	0	0	89607
sp Q14738-3 2A5D_HUMAN;tr B4DS07	6	0	524270	0	299880	0	1171100	0
sp Q13724-2 MOG5_HUMAN;tr Q58F0	6	0	523960	126710	0	312220	0	0
tr A0A024R582 A0A024R582_HUMAN;s	6	0	517530	181390	1851840	0	6526700	208460
sp Q9UIG0-2 BAZ1B_HUMAN;sp Q9UI0	6	0	497250	0	0	0	4103200	0
sp P42574 CASP3_HUMAN;tr C9JXR7	6	0	494820	0	1425000	0	5464100	453730
tr B4E205 B4E205_HUMAN;sp O95486	6	0	435410	0	1645130	0	0	112540
sp P62873-2 G8B1_HUMAN;tr B2R6K4	6	0	430980	198171	2065500	361117	2207041	350050
tr H3BLU7 H3BLU7_HUMAN;tr V9HWA	6	0	418000	1139700	2605300	824030	7273900	1527900
sp Q3YEC7 RABL6_HUMAN;sp Q3YEC7	6	0	416150	563700	1051700	219420	2283400	298110
sp Q15750-2 TAB1_HUMAN;tr A8K6K3	6	0	399130	0	2786100	0	4445200	0
tr B4DZF1 B4DZF1_HUMAN;sp Q9UHD	6	0	393750	1409800	1555200	676970	3256500	3791205
sp P26232-3 CTNA2_HUMAN;sp P2623	6	0.004246	384990	0	0	0	0	0
tr B5MCF9 B5MCF9_HUMAN;sp O0054	6	0	384910	76239	312207	0	1985410	148260
sp Q9H1B7 I2BPL_HUMAN	6	0	382020	0	328320	0	5512120	140350
tr H7BXY3 H7BXY3_HUMAN;sp Q7L2E3	6	0	381170	0	651920	0	3936200	100210
sp Q96B26 EXOS8_HUMAN;tr Q5JXM0	6	0	375990	295690	1009400	227980	6252400	356320
sp Q6PJ69 TRI65_HUMAN;tr H0YGS7	6	0	373650	0	172930	0	4924300	0
tr A0A024R3P9 A0A024R3P9_HUMAN;s	6	0	342740	536010	1606500	477340	7099500	276660
sp P82979 SARNP_HUMAN;tr Q567R9	6	0	336000	831280	2905100	904770	12695375	3015000
sp Q12792 TWF1_HUMAN;sp Q12792-	6	0	333590	95497	502980	81355	1094400	151180
sp Q86TP1 PRUNE_HUMAN;sp Q86TP-	6	0	326350	611620	2519700	825770	5937260	937724
tr A0A024R565 A0A024R565_HUMAN;s	6	0	316970	1056600	2223860	376880	9940480	2275400
sp Q7Z5L9-2 I2BP2_HUMAN;sp Q7Z5L	6	0	316768	369390	3705700	824940	2620900	0
sp Q9NT62 ATG3_HUMAN;sp Q9NT62-	6	0	308395	0	0	166610	9225300	366620
sp Q06124-2 PTN11_HUMAN;sp Q061	6	0	305810	173850	1057991	0	2415900	0
sp Q8NB90 SPAT5_HUMAN;sp Q8NB9	6	0	299400	0	470670	0	4989614	0
sp Q8TC12 RDH11_HUMAN;tr G3V2G6	6	0	296760	382990	522230	145650	485080	0
sp P83881 RL36A_HUMAN;tr H0Y5B4	6	0	290010	325051	672070	323430	19126550	224850
tr Q6IPH7 Q6IPH7_HUMAN;tr E7EPB3	6	0	272650	261150	1368400	160460	15418000	625220
tr A0A024R784 A0A024R784_HUMAN;s	6	0	271580	0	216880	0	3316000	0
sp O14686 KMT2D_HUMAN;sp O14686	6	0	243020	0	0	0	3093000	1072200
tr Q9UES0 Q9UES0_HUMAN;tr A4D2J0	6	0	224827	192090	369530	0	1107170	224420
sp O00560-3 SDCB1_HUMAN;tr A0A02	6	0	222710	397800	1393100	254030	25158950	551000
tr B2RCM6 B2RCM6_HUMAN;sp Q965	6	0	212330	287490	1065200	157380	10306200	243139
tr B4DJ39 B4DJ39_HUMAN;sp O94842-	6	0	209130	66336	548990	440110	9872800	332230
tr B2R802 B2R802_HUMAN;sp P09012	6	0	184830	327140	379080	0	1587300	317480
tr A2VCR0 A2VCR0_HUMAN;sp Q1569	6	0	175470	507590	1676370	262810	8568000	3045190
sp Q8IYS1 P20D2_HUMAN	6	0	174190	0	414420	0	0	541620
tr A0A024R7W9 A0A024R7W9_HUMAN	6	0	174170	648330	1237710	366260	5705400	388080
tr A0A024R713 A0A024R713_HUMAN;sp	6	0	156980	0	193890	593330	1184100	514130
tr Q53YD8 Q53YD8_HUMAN;sp P3640	6	0	138270	342640	2006300	558100	8695400	0

tr J3KTF8 J3KTF8_HUMAN;tr V9HWE8	6	0	118880	131790	506260	524300	0	1777960
tr X6RJS7 X6RJS7_HUMAN;sp P46736	6	0	117450	0	613520	0	7270200	195870
tr F5H5V4 F5H5V4_HUMAN;tr F5GX23	6	0	109060	0	34425	0	2471400	0
tr Q53HT6 Q53HT6_HUMAN;tr Q53G72	6	0	41999	453750	1221400	409870	147730	0
sp A2RTX5-2 SYTC2_HUMAN;sp A2RTX5	6	0.002737	0	0	0	0	1912200	0
sp O00560-2 SDCB1_HUMAN;tr G5EAC0	6	0	0	0	0	0	2945500	0
sp P15374 UCHL3_HUMAN;tr A0A087V6	6	0	0	0	0	0	1658000	0
sp P51617-4 IRAK1_HUMAN;sp P51617	6	0	0	0	1381200	0	4426600	0
sp Q08AD1-2 CAMP2_HUMAN;sp Q08AD1	6	0	0	0	0	0	6184100	0
sp Q5TB81-2 RNH2B_HUMAN;sp Q5TB81	6	0	0	0	0	0	3934600	0
sp Q7KZ85 B3KP6H_HUMAN;sp Q7KZ85	6	0	0	0	0	0	510960	0
sp Q8TDN6 BRX1_HUMAN;tr A0JLQ5	6	0	0	0	2737100	0	9346100	0
sp Q9HAU5 RENT2_HUMAN;sp Q9HAU5	6	0	0	0	0	0	3964990	0
sp Q9NZN3 EHD3_HUMAN	6	1	0	0	1959500	0	0	0
sp Q9P1Y5 CAMP3_HUMAN;tr D6W64	6	0	0	0	0	0	3194100	0
tr A0A024QZ77 A0A024QZ77_HUMAN;	6	0	0	0	0	0	204340	0
tr A0A024R861 A0A024R861_HUMAN;s	6	0	0	0	0	0	3841500	0
tr A0A024R9E6 A0A024R9E6_HUMAN;s	6	0	0	0	0	0	4782300	0
tr B3KPR5 B3KPR5_HUMAN;sp Q7Z2Z2	6	0	0	0	0	0	5411400	0
tr E7EVE9 E7EVE9_HUMAN;tr A8MX75	6	0	0	0	0	0	2804080	0
tr HOY990 HOY990_HUMAN	6	0.006526	0	0	1064100	0	2776300	0
tr K7EM56 K7EM56_HUMAN;tr S4R417	6	0	0	0	176290	0	4970900	0
tr Q56VW8 Q56VW8_HUMAN;sp P631	6	0	0	0	381080	0	0	0
tr Q59GC1 Q59GC1_HUMAN;sp Q0678	6	0	0	0	0	0	2748365	0
tr Q6IB29 Q6IB29_HUMAN;sp Q99848	6	0	0	0	0	0	6695100	0
tr Q6UUU9 Q6UUU9_HUMAN;tr G3V1F	6	0	0	0	276900	0	0	0
tr C9IQV0 C9IQV0_HUMAN;sp Q9BRU6	6	0	0	0	524670	0	14124000	598970
tr Q53F02 Q53F02_HUMAN;sp Q9UKF6	6	0	0	0	504190	0	6686800	318780
sp Q9UBF2-2 COPG2_HUMAN;sp Q9UBF2	6	0.000361	0	0	0	0	1089500	52243
tr A0A087VWS1 A0A087VWS1_HUMAN;	6	0	0	0	761560	0	5966400	301060
tr A0A024R3L9 A0A024R3L9_HUMAN;s	6	0	0	0	1445800	0	1166800	85179
tr B4DW90 B4DW90_HUMAN;tr Q53HI	6	0	0	0	0	0	3305700	443580
tr B4DDL4 B4DDL4_HUMAN;sp P30085	6	0	0	0	0	0	2806700	408780
tr B4E345 B4E345_HUMAN;tr Q5T7A9	6	0	0	0	235170	0	512910	163870
tr Q6FHM2 Q6FHM2_HUMAN;sp P628	6	0.000356	0	0	0	0	336870	134930
sp Q9Y281-3 COF2_HUMAN;tr Q549NC	6	0.000368	0	0	0	0	895660	375790
tr Q59GY3 Q59GY3_HUMAN;sp Q1324	6	0	0	0	935570	0	2664870	1736120
sp P30044 PRDX5_HUMAN;tr V9HW35	6	0	0	0	0	0	771860	607260
sp O60361 NDK8_HUMAN	6	1	0	0	109220	0	0	303380
sp Q969Q0 RL36L_HUMAN;tr B2R4V2	6	0	0	67874	88407	0	1686900	0
sp Q9NP55-2 BPIA1_HUMAN;sp Q9NP55	6	0	0	85819	0	0	9701000	0
sp Q9Y570 PPME1_HUMAN;sp Q9Y570	6	0	0	147810	992550	0	0	0
tr A4D275 A4D275_HUMAN;sp O15143	6	0	0	54378	58553	0	6866880	0
tr A0A024R649 A0A024R649_HUMAN;s	6	0	0	141460	724530	0	19914300	359630
tr F5H669 F5H669_HUMAN;tr B4DGF8	6	0	0	179690	830490	0	2072800	311070
tr A0A024R324 A0A024R324_HUMAN;s	6	1	0	158530	433520	0	1651000	328000
tr B2RD36 B2RD36_HUMAN;sp Q96DG	6	0	0	48255	0	0	1228900	244900
sp Q9UBQ0 VPS29_HUMAN;sp Q9UBC	6	0	0	245490	1512060	0	1603900	506410
tr A0A024QZN2 A0A024QZN2_HUMAN	6	0	0	228150	0	0	2182200	1258780
tr Q9NWX4 Q9NWX4_HUMAN;sp Q9NWX4	6	0	0	294578	697620	0	435510	256940
tr J3KP15 J3KP15_HUMAN;tr Q53FN0	6	0	0	130560	0	0	281680	407422
tr A0A024QZV0 A0A024QZV0_HUMAN	6	0	0	88937	0	0	0	187131
tr Q6IBA2 Q6IBA2_HUMAN;sp P53999	6	0	0	260740	0	0	0	1504000
sp Q9BUL8 PDC10_HUMAN;tr C9J5C3	6	0	0	165140	612650	196630	3361400	311220
tr Q6FIS1 Q6FIS1_HUMAN;tr Q6FHS4	6	0	0	0	1312290	640670	5630200	1629400
tr B8ZZU8 B8ZZU8_HUMAN;sp Q15370	6	0	0	136700	403560	273320	4339500	479710
sp Q96M27 PRRC1_HUMAN;sp Q96M27	6	0	0	1007574	4314400	3135088	1041700	12989580
tr Q69YJ7 Q69YJ7_HUMAN;tr B3KQH4	6	0	0	169640	151650	331270	2602600	734890
sp P28838-2 AMPL_HUMAN;tr V9HW3	6	0	0	0	0	162500	944670	0
tr Q6N093 Q6N093_HUMAN;sp P01851	6	0	0	284370	0	857670	4094100	1176350
tr A0A087WYL9 A0A087WYL9_HUMAN	6	0.009094	0	4802810	0	3695650	1391700	7278220
sp P28070 PSB4_HUMAN;tr B4DFL3 B4DFL3	6	0	0	382306	0	274312	117200	1033257
sp Q562R1 ACTBL_HUMAN	5	0.005475	135348459	3946500	171760000	0	81121000	12743500
tr Q7Z4W8 Q7Z4W8_HUMAN;sp P3526	5	0	16750140	13681020	28466070	8813110	180270000	25332550
sp P47813 IF1AX_HUMAN;sp O14602	5	0	7188150	1602300	11502000	1540700	48224000	3756000
sp P17900 SAP3_HUMAN;tr Q14427 C	5	0	6522502	372503	0	359557	360270	401800
sp Q9GZ29 UBA5_HUMAN;sp Q9GZ29	5	0	5760270	967520	3669585.3	186460	6399723	353562
sp Q01844-6 EWS_HUMAN;tr B0QYK0	5	0	5617380	1540050	8902540	1164180	52153530	2634550
sp Q12824-2 SNF5_HUMAN;sp Q12824	5	0	4950900	683960	4903100	0	11355660	1131500
sp P31689-2 DNJA1_HUMAN;sp P31689	5	0	4905900	2084600	11491000	2954100	13856000	4914640
tr J3QKS7 J3QKS7_HUMAN;sp Q969G3	5	0	3420800	0	1723900	0	4113800	251540
sp O14929 HAT1_HUMAN;sp O14929-2	5	0	3413000	1231200	4936800	854640	1363000	948015
sp P21964-2 COMT_HUMAN;sp P21964	5	0	2788100	234410	177120	0	4802800	264660
sp P24752 THIL_HUMAN;tr E9PRQ6 E9PRQ6	5	0	2602400	276930	3168600	349270	0	0
tr A8K8K1 A8K8K1_HUMAN;sp O00442	5	0	2524100	243720	5711033	520790	9030500	1754488
tr A0A024RDH8 A0A024RDH8_HUMAN	5	0	2406442	490710	3327880	573709	37178547	3007180

tr HOYE97 HOYE97_HUMAN;tr HOYE97	5	0	2400400	620520	5935600	0	7766500	0
tr Q53EY9 Q53EY9_HUMAN;sp Q8NEZ1	5	0	2322900	0	2835200	268480	8973900	1572900
sp P35613-3 BASI_HUMAN;tr A0A087V	5	0	2294700	0	3408810	0	751170	0
tr B4DWR5 B4DWR5_HUMAN;sp P074	5	0	2266520	0	0	0	1493070	0
tr A8K5M4 A8K5M4_HUMAN;sp Q131	5	0	2137770	0	0	0	0	0
sp Q96J01 THOC3_HUMAN;sp Q96J01	5	0	1992000	0	4474100	0	6267670	0
tr K7EK77 K7EK77_HUMAN;sp P25705	5	0	1936200	980040	2065070	441667	2723540	0
tr A0A024RAE4 A0A024RAE4_HUMAN;	5	0	1933097	1704600	34478000	883370	10143000	757410
tr B3KRC6 B3KRC6_HUMAN;sp Q16850	5	0	1886700	263370	391140	0	0	0
sp Q9NXR7-4 BRE_HUMAN;sp Q9NXR7	5	0	1867300	0	5630300	0	14592000	0
tr K7EQ03 K7EQ03_HUMAN;sp Q9BTD	5	0	1852400	229780	3402300	686260	17374000	2095800
tr Q1ET65 Q1ET65_HUMAN;tr Q1ET64	5	0	1823360	4982700	1852550	1661630	5760410	3561160
sp Q9HDC9-2 APMAP_HUMAN;sp Q9H	5	0	1783600	2003800	97133	118450	0	389670
tr E5RHG8 E5RHG8_HUMAN;tr A0A024	5	0	1708110	3213140	1275540	5441100	2858800	1632790
tr A8MYT4 A8MYT4_HUMAN;sp Q8NE	5	0	1617000	275200	1107600	262070	6502300	228600
sp Q6NUQ4-2 TM214_HUMAN;tr B3LE	5	0	1575120	332190	864120	401760	0	0
tr Q53FS4 Q53FS4_HUMAN;tr B2R774	5	0	1557800	474990	2824800	0	7390500	658910
tr Q96RG4 Q96RG4_HUMAN;sp Q9Y4H	5	0	1533600	176460	1658800	0	5136500	137540
sp P81605-2 DCD_HUMAN	5	0.002677	1521030	159680	1344420	1887930	0	1095500
sp O95235-2 KI20A_HUMAN;sp O9523	5	0	1519300	325920	1127700	88132	0	0
sp Q9Y5K6 CD2AP_HUMAN	5	0	1493800	0	3019900	0	1578500	0
tr B3KN05 B3KN05_HUMAN;tr B2RBE0	5	0	1473500	0	2413200	136860	0	0
sp Q96EK5 KBP_HUMAN	5	0	1468300	309460	955320	968360	6152900	962390
sp Q08378-2 GOGA3_HUMAN;sp Q083	5	0	1458600	0	1792930	0	733010	0
sp Q9UN51-2 TIM_HUMAN;sp Q9UN5	5	0	1401300	0	0	0	2579100	0
sp Q9BQC3 DPH2_HUMAN;sp Q9BQC3	5	0	1399800	0	2526300	0	2960800	0
tr F8VQE1 F8VQE1_HUMAN;sp Q9UHE	5	0	1335310	0	3013040	0	5998100	291550
sp Q9NQ15 EXOS3_HUMAN;sp Q9NQ1	5	0	1316580	770930	2022000	406380	26442750	1602200
sp P25788-2 PSA3_HUMAN;tr Q6IB71	5	0	1242260	638864	0	315350	2033800	910970
sp Q3MHD2 LSM12_HUMAN;sp Q3MH	5	0	1178200	1357900	3515700	959420	24072000	1167500
sp Q9BVI4 NOC4L_HUMAN;tr F5H303	5	0	1176000	0	0	0	816340	0
tr Q6FGM6 Q6FGM6_HUMAN;tr E5KN	5	0	1026400	0	2696200	754660	2696500	1405775
tr B3KUR4 B3KUR4_HUMAN;tr A4D295	5	0	997790	153650	0	125050	2777500	173160
sp Q03252 LMNB2_HUMAN;tr J9JID7	5	0	969250	0	1060300	0	0	0
tr E7EX83 E7EX83_HUMAN;tr E7ENQ1	5	0	951940	0	382190	0	3367000	0
sp O60784-4 TOM1_HUMAN;sp O6078	5	0	937050	0	3038800	316260	5523300	418700
tr A0A024RC00 A0A024RC00_HUMAN;s	5	0	922180	292710	0	0	2518900	382040
tr Q6IAX2 Q6IAX2_HUMAN;sp P46778	5	0	920990	1127900	3866295	635200	54491380	1946180
tr H7BYG8 H7BYG8_HUMAN;sp O9482	5	0	908470	0	337110	0	3380330	0
sp Q9UBB6-2 NCDN_HUMAN;sp Q9UB	5	0	881510	326290	1456200	348030	4389300	361090
tr A0A024R613 A0A024R613_HUMAN;sp	5	0	862167	614210	997830	277790	14654240	508920
sp P00387 NB5R3_HUMAN;sp P00387	5	0	856460	727290	924610	0	1051100	277810
tr Q6IBH6 Q6IBH6_HUMAN;sp P61254	5	0	851170	367650	2314000	568610	31350100	1517300
tr Q53FG6 Q53FG6_HUMAN;sp Q1542	5	0	844050	298378	1045250	0	5461500	1453300
sp Q9UK59 DBR1_HUMAN;tr B3KSR5	5	0	837920	291050	4684300	377160	15486350	307310
sp Q9Y4C2-2 F115A_HUMAN;sp Q9Y4C	5	0	836070	0	161070	0	1546300	0
sp Q96I15 SCLY_HUMAN;tr A0A0A0M0	5	0	790080	0	0	0	1355000	0
tr A4D2P2 A4D2P2_HUMAN;tr A4D2P1	5	0	788050	1584400	938670	344010	1896540	918455
tr C9J4Z3 C9J4Z3_HUMAN;sp P61513	5	0	775020	450660	3022130	145520	22982000	1569900
sp Q12800-2 TFCP2_HUMAN;sp Q1280	5	0	732000	440350	4310400	647940	11702800	490780
sp Q9H974-3 QTRD1_HUMAN;sp Q9H9	5	0	730060	0	3582700	967220	2638600	476210
sp Q9UPY3-2 DICER_HUMAN;sp Q9UP	5	0	723300	59681	155100	0	5189400	0
tr C9J0K6 C9J0K6_HUMAN;sp P30626-1	5	0	712308	390870	1192600	559150	4507800	5222640
tr A0A087WY55 A0A087WY55_HUMAN	5	0	698353	1012770	1948700	720759	14073000	3756618
sp Q99961-3 SH3G1_HUMAN;tr Q9UQU	5	0	681060	0	3471400	550629	4800500	1856039
tr F8WCX2 F8WCX2_HUMAN;sp Q8N1	5	0	679710	0	270220	103250	1710600	0
tr A0A024R912 A0A024R912_HUMAN;s	5	0	649520	66616	846900	187040	6515700	902380
tr B3KPP7 B3KPP7_HUMAN;tr B4DHJ9	5	0	572190	0	849850	0	1336300	0
sp Q15386 UBE3C_HUMAN;tr B4DHJ9	5	0	545680	0	0	0	5022400	0
tr A0A024RBA9 A0A024RBA9_HUMAN;	5	0	524980	337000	1038800	196570	3101100	266880
tr I3L2K5 I3L2K5_HUMAN;sp Q8IWR0	5	0	524380	0	158950	0	3574500	0
tr B2R7D3 B2R7D3_HUMAN;sp Q96D7	5	0	516780	0	95725	0	4709800	0
sp P51571 SSRD_HUMAN;tr A6NLM8	5	0	505150	104630	240760	97464	1649900	0
sp P37108 SRP14_HUMAN;tr HOYLA2	5	0	495390	681240	3813700	1761429	14804000	5243450
tr A0A024R1T5 A0A024R1T5_HUMAN;s	5	0	478710	296410	2669900	215670	3921700	179140
sp Q9H7D7-2 WDR26_HUMAN;sp Q9H	5	0	431290	0	41434	0	1402400	119040
tr Q53FL4 Q53FL4_HUMAN;tr A0A087V	5	0	424750	0	0	0	3545400	0
tr Q6IB11 Q6IB11_HUMAN;sp O00264	5	0	424650	246800	742730	0	6330100	0
sp P53990-2 IST1_HUMAN;tr A8K5S3	5	0	424220	212450	2949500	232720	9242700	208850
tr Q5T8C6 Q5T8C6_HUMAN;sp Q13042	5	0	407640	0	0	0	3429220	0
tr A0A0A0MR59 A0A0A0MR59_HUMAN	5	0.006544	400630	0	0	0	496880	0
sp O95219 SNX4_HUMAN;sp O95219-1	5	0	399070	261270	406790	0	772850	0
sp Q9H444 CHM4B_HUMAN;sp Q96CF	5	0	398450	0	453580	0	258960	83268
sp O75569-3 PRKRA_HUMAN;sp O755	5	0	373490	0	714850	64244	17023000	756330
tr A8K7H4 A8K7H4_HUMAN;sp O43921	5	0	368420	0	2987690	129150	7502700	1015800



tr B3KY28 B3KY28_HUMAN;tr A8K968	5	0	362480	0	466010	0	0	0
tr Q53HI2 Q53HI2_HUMAN;sp Q9UJV9	5	0	346240	81487000	652430	0	4263800	0
sp Q9BRP1 PDD2L_HUMAN;tr U3KQA4	5	0	331400	0	2037200	108070	6066186	321220
sp Q9UID3 VP551_HUMAN;sp Q9UID3	5	0	307460	0	1568400	0	3105400	0
sp Q9NXV6 CARF_HUMAN;tr B3K7W3	5	0	300760	0	371610	0	877660	84895
sp Q5VIR6-4 VP553_HUMAN;tr B3K506	5	0	249790	0	219250	0	4090212	0
sp Q13526 PIN1_HUMAN;tr K7EN45 K	5	0	235270	0	422600	231760	8921900	544250
tr Q5T321 Q5T321_HUMAN;sp Q8NFP6	5	0.003308	220020	0	152270	0	304290	0
tr A0A024R4X1 A0A024R4X1_HUMAN;tr	5	0	213190	0	872870	0	2522300	19536
tr C9J9W2 C9J9W2_HUMAN;tr A0A024	5	0	190320	195320	774660	159610	5645900	309910
tr X5D7P8 X5D7P8_HUMAN;tr Q6E0B2	5	0	181600	0	1683200	0	4484000	0
tr C9JEL3 C9JEL3_HUMAN;tr B9A044 E	5	0	180650	0	1377050	0	7878900	0
tr A0A024R1Q3 A0A024R1Q3_HUMAN;tr	5	0	171990	759340	1659070	0	4587700	0
tr F5CTF3 F5CTF3_HUMAN;sp P27707	5	0	167230	0	440090	0	5296100	234240
sp Q13242 SRSF9_HUMAN;tr A8K3M9	5	0	150090	0	0	0	4813900	261310
tr H9A532 H9A532_HUMAN;sp Q6W2J	5	0	149290	0	105460	0	1001600	0
sp Q9H7Z7 PGES2_HUMAN;tr B3KPP2	5	0	140150	267270	573890	0	4844100	120590
tr Q5QPL9 Q5QPL9_HUMAN;sp Q9UKF	5	0	129540	178000	145140	0	4289200	0
sp Q6ZNI7 LN28B_HUMAN;sp Q6ZNI7	5	0	83911	0	476240	0	5166000	69419
tr J3KRC4 J3KRC4_HUMAN;tr V9HWF3	5	0	70310	0	0	0	9623100	0
sp O60493 SNX3_HUMAN;sp O60493-4	5	0	70308	0	78575	0	251260	0
tr B2R823 B2R823_HUMAN;sp Q9NRX	5	0	52023	0	0	0	2293400	0
sp O00287 RFXAP_HUMAN	5	0	0	0	0	0	2450300	0
sp O75113 N4BP1_HUMAN;tr B3KMA4	5	0	0	0	0	0	4339100	0
sp P32929-3 CGL_HUMAN;sp P32929 C	5	0	0	0	456570	0	0	0
sp P41227 NAA10_HUMAN;sp P41227	5	0	0	0	0	0	659690	0
sp P62314 SMD1_HUMAN;tr Q7Z5A3 C	5	0	0	0	166030	0	13565000	0
sp Q12872 SFSWA_HUMAN;sp Q12872	5	0	0	0	265690	0	3603960	0
sp Q15417-2 CNN3_HUMAN;tr E9PDU	5	0	0	0	122400	0	1012200	0
sp Q15650 TRIP4_HUMAN;tr H0YL91 H	5	0	0	0	0	0	4878300	0
sp Q86U06-5 RBM23_HUMAN;tr Q6IA5	5	0	0	0	0	0	1066900	0
sp Q8NFB4 NUP37_HUMAN;tr B4DKV	5	0	0	0	359940	0	2738200	0
sp Q93034 CUL5_HUMAN;tr L0L6C1 LC	5	0	0	0	883590	0	3691360	0
sp Q96CW1-2 AP2M1_HUMAN;tr A0A	5	0	0	0	1234195	0	4741500	0
sp Q9NR12-2 PDL17_HUMAN;sp Q9NR	5	0	0	0	1122900	0	5120400	0
sp Q9NSI2-2 F207A_HUMAN;sp Q9NSI	5	0	0	0	105730	0	1720100	0
sp Q9NTJ5 SAC1_HUMAN;tr E9PGZ4 E	5	0	0	0	348550	0	0	0
sp Q9UGJ1-2 GCP4_HUMAN;sp Q9UGJ	5	0	0	0	1050300	0	2477000	0
sp Q9UNH7-2 SNX6_HUMAN;tr B4DJ5	5	0	0	0	0	0	0	0
sp Q9UNX3 RL26L_HUMAN;tr E5RIT6 I	5	0	0	0	0	0	403860	0
sp Q9Y316-2 MEMO1_HUMAN;sp Q9Y	5	0	0	0	189470	0	2823700	0
sp Q9Y4W2-2 LAS1L_HUMAN;sp Q9Y4	5	0	0	0	0	0	5205780	0
tr A0A024R294 A0A024R294_HUMAN;sp	5	0	0	0	245050	0	3963200	0
tr A0A024R3A2 A0A024R3A2_HUMAN;sp	5	0	0	0	0	0	2925100	0
tr A0A024R8R4 A0A024R8R4_HUMAN;sp	5	0	0	0	0	0	4377000	0
tr A0A024RAL3 A0A024RAL3_HUMAN;sp	5	0	0	0	0	0	5116500	0
tr A4FU51 A4FU51_HUMAN;sp Q6N06	5	1	0	0	174650	0	777880	0
tr B2R5U7 B2R5U7_HUMAN;tr A0A087	5	0	0	0	0	0	3035500	0
tr B3KM97 B3KM97_HUMAN;sp Q9NZ	5	0	0	0	227010	0	337410	0
tr B3KMP2 B3KMP2_HUMAN;sp Q9UJ	5	0	0	0	944390	0	5599100	0
tr B3KQF0 B3KQF0_HUMAN;sp Q9UGF	5	0	0	0	1764000	0	0	0
tr B3KRJ9 B3KRJ9_HUMAN;sp Q8WXA	5	0	0	0	629020	0	11048990	0
tr B4DNR3 B4DNR3_HUMAN;tr V9HW	5	0	0	0	0	0	6570600	0
tr B4DXV1 B4DXV1_HUMAN;sp Q9H9T	5	0	0	0	1286500	0	7479100	0
tr B7ZA76 B7ZA76_HUMAN;tr B4DIA9	5	0	0	0	900600	0	1922000	0
tr C9IYN7 C9IYN7_HUMAN;tr B4DP60	5	0	0	0	0	0	1924400	0
tr D6RHE1 D6RHE1_HUMAN;tr I6L894	5	0	0	0	0	0	3802500	0
tr E7ER60 E7ER60_HUMAN;tr B4DLC4	5	0	0	0	0	0	3692300	0
tr E9PGC0 E9PGC0_HUMAN;tr B4DTX4	5	0	0	0	345310	0	2504100	0
tr F5H3Y4 F5H3Y4_HUMAN;sp Q8IY37	5	0	0	0	0	0	2243500	0
tr F6WIT2 F6WIT2_HUMAN;sp Q15257	5	0	0	0	0	0	231840	0
tr G3V1S1 G3V1S1_HUMAN;tr B3KTH1	5	0	0	0	0	0	2095000	0
tr I3L412 I3L412_HUMAN;tr K7ENA6 K	5	0	0	0	0	0	3353030	0
tr I7GPQ7 I7GPQ7_HUMAN;sp Q92541	5	0	0	0	0	0	1877400	0
tr J3KPD3 J3KPD3_HUMAN;tr I3L521 I	5	0	0	0	0	0	3191000	0
tr MOR088 MOR088_HUMAN;tr E9PCT1	5	0	0	0	0	0	963910	0
tr Q1W6H1 Q1W6H1_HUMAN;sp P293	5	0	0	0	0	0	1464000	0
tr Q5TOF3 Q5TOF3_HUMAN;sp Q13823	5	0	0	0	0	0	3742800	0
tr Q5VU11 Q5VU11_HUMAN;sp P7834	5	0	0	0	0	0	1105100	0
tr Q6FGR6 Q6FGR6_HUMAN;sp P1938	5	0	0	0	102650	0	5114500	0
tr Q8NI62 Q8NI62_HUMAN	5	1	0	0	0	0	2705500	0
tr V9HWCS V9HWCS_HUMAN;tr F5GM	5	0	0	0	0	0	3219000	0
sp Q9UQRO SCML2_HUMAN;tr B4DZR	5	0	0	0	619050	0	4343800	55666
tr A0A024RDY9 A0A024RDY9_HUMAN;tr	5	0	0	0	1316300	0	3782600	187830

tr B4DEQ6 B4DEQ6_HUMAN;sp Q6ZW	5	0	0	0	0	0	0	3083300	174180
sp Q9UJW0 DCTN4_HUMAN;sp Q9UJW	5	0	0	0	823920	0	0	4665300	382310
tr B4DQC7 B4DQC7_HUMAN;tr A8K6D	5	0	0	0	186570	0	0	1541400	133010
tr A0A024R1X3 A0A024R1X3_HUMAN;s	5	0	0	0	133990	0	0	4582900	509240
tr A0A024R2X1 A0A024R2X1_HUMAN;s	5	0	0	0	1131400	0	0	2309800	387840
sp Q9Y5S9-2 RBM8A_HUMAN;tr A0A0	5	0	0	0	245420	0	0	5765600	1066400
sp Q9NPD3 EXOS4_HUMAN;tr E9PI41	5	0	0	0	0	0	0	893240	394140
sp P20290 BTF3_HUMAN;sp P20290-2	5	0	0	0	0	0	0	711120	822270
tr A4D177 A4D177_HUMAN;sp Q13185	5	0	0	0	104050	0	0	627770	1261700
sp Q13404 UB2V1_HUMAN;sp Q13404	5	0	0	0	0	0	0	404590	830790
sp Q43598 DNPH1_HUMAN;tr H0Y8X4	5	0	0	0	0	0	0	518330	1258000
sp Q9H3K6 BOLA2_HUMAN;sp Q9H3K	5	0	0	0	0	0	0	322230	5224800
tr B4DR80 B4DR80_HUMAN;tr Q6P0Y1	5	0.002718	0	0	198590	0	0	0	226590
tr D6RG15 D6RG15_HUMAN;sp Q6IB50	5	0	0	0	451710	0	0	0	334560
sp Q5T200 ZC3HD_HUMAN;sp Q5T200	5	0	0	725290	0	0	0	777000	0
sp Q709F0 ACD11_HUMAN;tr D6RD18	5	0	0	269660	555740	0	0	3111300	0
sp Q86YR5-4 GPSM1_HUMAN;tr A0A0	5	0	0	46255	186830	0	0	2941613	0
tr B7Z3B5 B7Z3B5_HUMAN;sp Q15349	5	0.006579	0	74830	0	0	0	1063000	0
tr Q3SYF1 Q3SYF1_HUMAN;sp Q9UMY	5	0	0	49220	0	0	0	0	0
tr Q59H56 Q59H56_HUMAN;tr Q5SRN	5	0	0	698810	292180	0	0	3620700	0
tr A0A024R3V8 A0A024R3V8_HUMAN;	5	0	0	125460	0	0	0	3413900	58696
sp Q9Y3B2 EXOS1_HUMAN;tr R4GMQ	5	0	0	106330	797200	0	0	8179000	152970
sp Q9H2P9-3 DPH5_HUMAN;sp Q9H2F	5	0	0	367820	1035600	0	0	7522100	199270
tr B7Z1J9 B7Z1J9_HUMAN;tr B7ZKW4	5	0	0	368190	295420	0	0	9096600	297830
sp Q9Y3B3-2 TMED7_HUMAN;sp Q9Y3	5	0	0	106620	0	0	0	6681800	243960
sp P11441 UBL4A_HUMAN;tr Q5HY81	5	0	0	248670	508590	0	0	3431800	436340
sp Q92688-2 AN32B_HUMAN;tr Q53F3	5	0	0	250680	0	0	0	8584400	1955500
sp P49458 SRP09_HUMAN;tr Q6P250	5	0	0	30745	0	0	0	2553638	1624700
tr B4DMK6 B4DMK6_HUMAN;sp Q9NF	5	0	0	116030	1380300	111410	0	4909800	0
tr B4DXX1 B4DXX1_HUMAN;tr B2R713	5	0	0	0	759720	86730	0	1566990	467950
sp Q08209-3 PP2BA_HUMAN;sp Q082	5	0	0	886490	2674760	547850	0	8682040	969160
tr Q6FI97 Q6FI97_HUMAN;tr Q53F50	5	0	0	243560	955940	226530	0	2696700	300590
tr A8K4M5 A8K4M5_HUMAN;sp Q6056	5	0	0	0	111490	36322	0	2010000	0
tr Q6FGV9 Q6FGV9_HUMAN;sp Q1512	5	0	0	162840	406860	140170	0	5126300	566390
sp Q5VWZ2 LYPL1_HUMAN;sp Q5VW2	5	0	0	195380	422500	157780	0	3623400	230580
tr B2R9D9 B2R9D9_HUMAN;sp Q96EK	5	0	0	0	527150	214500	0	3841700	0
tr H6V745 H6V745_HUMAN;tr F5ATB8	5	0	0	0	158990	444480	0	1816190	0
tr A0A024R258 A0A024R258_HUMAN;s	5	0	0	0	0	689600	0	2747900	1690300
tr B4DEA6 B4DEA6_HUMAN;sp Q4376	5	0	0	0	0	923810	0	0	1979460
tr Q502X2 Q502X2_HUMAN;sp Q9NR2	5	0	0	0	0	334040	0	0	570610
tr Q6NS95 Q6NS95_HUMAN;tr A0M8C	5	0.0007	0	414770	0	689000	59202	595501	0
tr A3KPC7 A3KPC7_HUMAN;sp Q9987	4	0	32146500	6046110	51236880	2474674	71762780	11824260	0
sp P60866 RS20_HUMAN;sp P60866-2	4	0	16619840	14213108	34961760	10491680	160110480	22249760	0
tr Q9BRL5 Q9BRL5_HUMAN;tr B4DJ51	4	0	14019900	1574540	3999300	1048100	11387620	3510700	0
sp P27482 CALL3_HUMAN	4	0	13751140	291500	508460	251950	1476800	575200	0
tr I4AY87 I4AY87_HUMAN;sp P14174	4	0	10185450	9585060	18677890	7261070	68936810	23623960	0
tr B2R4C5 B2R4C5_HUMAN;sp P61626	4	0	9453400	1613130	2217910	3680430	4581800	4248910	0
sp Q5VW32-2 BROX_HUMAN;sp Q5VV	4	0	5621400	2888800	8728503	2237400	3254200	1580600	0
sp A6NHL2-2 TBAL3_HUMAN;sp A6NH	4	0	5365100	1303000	7626700	2211500	27583000	5600040	0
sp P62851 RS25_HUMAN	4	0	4790230	5696980	19619340	3920814	65443940	15921130	0
sp Q5TAX3 TUT4_HUMAN;tr H0YDJ1	4	0	4777400	0	5231400	0	10387000	0	0
sp Q9NZH8-2 IL36G_HUMAN;sp Q9NZ	4	0	4689370	622670	761810	730220	269130	98307	0
sp P42677 RS27_HUMAN;tr Q5T4L4 Q	4	0	4056860	2807100	6954528	763696	64255000	2745539	0
tr E4W6B6 E4W6B6_HUMAN;tr B2R4D	4	0	3453630	1247985	11832570	2010398	112101380	7692010	0
tr A0A024R7B7 A0A024R7B7_HUMAN;s	4	0	3206307	2148158	8971224	1298300	12081590	4743765	0
tr Q8WUB5 Q8WUB5_HUMAN;tr Q96J	4	0	2895400	318540	4377960	497690	1644530	806060	0
tr J3KRG2 J3KRG2_HUMAN;sp Q96QA	4	0	2518380	0	0	270930	0	0	0
tr B2R4C1 B2R4C1_HUMAN;sp P62899	4	0	2347770	1444065	5431010	907693	51462310	5176010	0
sp Q6ZVX7 FBX50_HUMAN	4	0	2271720	1280100	698460	2427220	1187550	0	0
sp P42766 RL35_HUMAN;tr F2Z388 F2	4	0	2265420	955467	4576180	365240	43998900	3620660	0
tr A8K6V7 A8K6V7_HUMAN;tr A0A024	4	0	2118600	576680	4173344	311910	11066807	62543	0
tr A8K9V9 A8K9V9_HUMAN;sp Q96KN	4	0	2099700	283050	3353100	611830	109240	0	0
sp P21796 VDAC1_HUMAN;tr B4DEI3	4	0	1995740	121960	717630	0	476560	0	0
tr A0A024RB14 A0A024RB14_HUMAN;s	4	0	1985040	2235326	5869700	1619930	82863560	12662400	0
sp P49411 EFTU_HUMAN	4	0	1822000	241710	585400	0	0	0	0
sp Q9NWW4 CA123_HUMAN;tr D3DQ3	4	0	1795630	1651400	3200800	1057800	38446000	1217600	0
tr A0A024QZX3 A0A024QZX3_HUMAN;	4	0	1775100	265489000	4826220	304747000	5007920	448075000	0
tr B4DYH4 B4DYH4_HUMAN;sp Q1467	4	0	1691000	0	0	0	526350	0	0
tr A0A087WZH7 A0A087WZH7_HUMAN	4	0	1551700	1551200	2867500	840640	599910	1283680	0
tr B0I1S0 B0I1S0_HUMAN;sp Q8NCM8	4	0	1421100	0	3356541	0	1241200	0	0
sp Q6NXE6-2 ARMC6_HUMAN;sp Q6N	4	0	1385900	0	2239800	0	8462400	0	0
tr V9HW44 V9HW44_HUMAN;sp P684	4	0	1353000	194740	531850	411100	2723500	2327900	0
sp Q86TG7-2 PEG10_HUMAN;tr A0A0	4	0	1346200	295700	1643334	291510	897630	0	0
sp P16383-2 GCFC2_HUMAN;sp P1638	4	0	1274900	129640	210120	0	2107700	206350	0
tr Q6FHM6 Q6FHM6_HUMAN;sp P557	4	0	1215000	2446800	3522900	1888700	21444000	3683300	0
tr Q569I7 Q569I7_HUMAN;tr S6AWF4	4	0.004257	1210600	0	1317700	0	2748200	0	0

sp Q9H501 ESF1_HUMAN;tr A0JLU5 A	4	0	1098670	0	0	0	576230	0
sp P22528 SPR1B_HUMAN;tr Q2I377 C	4	0	1096160	741520	734230	738512	1041200	1554770
tr Q4W5L2 Q4W5L2_HUMAN;tr H6UY5	4	0	1092100	163990	1524200	0	503120	0
sp P49815-7 TSC2_HUMAN;sp P49815-	4	0	1089300	0	0	0	2896300	0
sp Q8N4C6-6 NIN_HUMAN;sp Q8N4C6-	4	0	1079800	0	429830	0	0	0
tr C7DJ52 C7DJ52_HUMAN	4	0	1079400	4818340	1651200	2859460	3263000	1002319
sp P19174 PLCG1_HUMAN;sp P19174-	4	0	995330	0	344600	0	0	291090
tr B4DQG8 B4DQG8_HUMAN;sp Q9NP	4	0	958200	0	1267900	0	1944700	0
sp P43487-2 RANG_HUMAN;sp P43487-	4	0	932440	964950	3085600	873010	9822200	909169
tr B4DJV5 B4DJV5_HUMAN;tr Q9BV37	4	0	929160	0	1444200	0	3843400	0
tr Q561W4 Q561W4_HUMAN;sp Q129	4	0	919300	0	2551900	0	4113800	806900
tr A7E2Y5 A7E2Y5_HUMAN;sp O75165-	4	0	878790	0	0	0	1470300	0
sp O14578 CTRO_HUMAN;sp O14578-	4	0	812090	0	410810	0	0	0
sp Q6VMQ6-2 MCAF1_HUMAN;sp Q6V	4	0	787770	237610	317540	153270	3403000	366850
sp P63092-3 GNAS2_HUMAN;tr Q5FW	4	0	772840	0	2446400	322833	1875020	0
sp Q14181 DPOA2_HUMAN;tr B3KSX6	4	0	747330	0	1190090	0	5018590	60629
sp Q9UKJ3-2 GPTC8_HUMAN;sp Q9UK	4	0	740200	0	418610	0	3733900	0
tr B4DJ85 B4DJ85_HUMAN;sp Q92990	4	0	718460	226220	3337100	250720	6961300	307214
tr Q6IAT9 Q6IAT9_HUMAN;sp P28072	4	0	706593	696050	695256	489230	1347540	1493390
tr H7C1I7 H7C1I7_HUMAN;sp Q5VZL5-	4	0	703290	0	578530	0	1721800	0
tr A8K6Q9 A8K6Q9_HUMAN;tr A0A024	4	0	684170	0	2712900	179270	0	0
tr A0A024R9M3 A0A024R9M3_HUMAN	4	0	676860	267740	625350	0	4959700	0
sp O75175-2 CNOT3_HUMAN;tr B7Z6J	4	0	651260	0	512420	0	6028400	23114
tr B4DZM3 B4DZM3_HUMAN;tr Q53FR	4	0	633870	144850	1004800	136400	8934100	534440
sp Q86W56-5 PARG_HUMAN;tr B4DX7	4	0	624380	0	0	0	1282700	98789
sp Q16512-3 PKN1_HUMAN;sp Q16512-	4	0	610050	0	591520	0	3294600	0
sp Q16637-4 SMN_HUMAN;sp Q16637-	4	0	604690	625290	797300	141150	13299000	0
tr Q4G0D9 Q4G0D9_HUMAN;tr B4DEN	4	0	598230	0	180450	0	0	0
sp Q96GM8 TOE1_HUMAN;tr B3KSC7	4	0	594970	0	1582630	0	4283700	0
tr Q504W7 Q504W7_HUMAN;sp Q019	4	0	575730	76189	858890	0	1030098	46159
tr A0A024R3R5 A0A024R3R5_HUMAN;s	4	0	559600	0	261581	0	0	0
tr A0A024RDE8 A0A024RDE8_HUMAN;	4	0	555090	0	439720	0	2958200	0
tr C9K025 C9K025_HUMAN;sp P18077	4	0	551389	1161468	3710510	651790	52779000	4274700
sp Q8WVB6-3 CTF18_HUMAN;tr E7EX	4	0	546740	0	0	0	2164800	0
tr Q32Q83 Q32Q83_HUMAN;tr Q6P39	4	0	540980	237370	732300	190780	5040200	416090
sp Q9NY33-4 DPP3_HUMAN;tr Q5JPB	4	0	527490	0	1441948	200210	585710	423786
tr E5K1K1 E5K1K1_HUMAN;tr E5K1J9 E	4	0	489360	97344	0	0	0	0
sp Q96DH6-2 MS12H_HUMAN;tr B4DH	4	0	463030	0	0	0	10307600	495259
tr C91LU1 C91LU1_HUMAN;sp P52434	4	0	445780	0	476530	0	215490	255640
sp Q53LP3 SWAHC_HUMAN	4	0	434480	169850	1252700	204080	1285000	0
sp Q99504-5 EYA3_HUMAN;sp Q99504-	4	0	424980	159350	1581700	201640	3114100	0
tr A0A024R3X7 A0A024R3X7_HUMAN;t	4	0.000363	412540	0	0	0	0	0
tr A0A090N7X1 A0A090N7X1_HUMAN;	4	0	409960	270330	274980	256950	4248000	0
sp Q92615 LAR4B_HUMAN;tr H0Y641	4	0	409210	0	471820	0	5483700	169240
tr H3BV55 H3BV55_HUMAN;tr B2R6D4	4	0	403860	0	811670	416690	11942000	715320
sp O00291-3 HIP1_HUMAN;tr V5LU97	4	0	396490	145807	87403	0	2532000	0
tr A0A075B785 A0A075B785_HUMAN;s	4	0	395430	0	0	0	1689900	0
sp Q92896 GSLG1_HUMAN;sp Q92896-	4	0	382250	0	552760	0	522670	0
tr A8KA83 A8KA83_HUMAN;sp Q9POL	4	0	376410	0	606380	0	0	175220
tr Q05DK5 Q05DK5_HUMAN;sp P3561	4	0	363360	0	0	0	3397610	0
tr Q5J8M4 Q5J8M4_HUMAN;sp P7840	4	0	350550	0	888180	0	6032000	618790
sp Q95372 LYPA2_HUMAN;tr Q5QPQ1	4	0	349031	0	47901	71490	4936870	0
sp P01877 IGHA2_HUMAN;tr Q9NPP6	4	0.003634	342170	0	0	371160	0	332590
sp Q8N573-5 OXR1_HUMAN;sp Q8N57	4	0	339930	0	341100	0	2217600	0
tr A8K3Y5 A8K3Y5_HUMAN;sp Q9NX5	4	0	331960	0	922560	0	2225200	428410
sp P22681 CBL_HUMAN	4	0	330740	0	0	0	2321600	0
tr F8VZJ2 F8VZJ2_HUMAN;tr F8WOW4	4	0	305050	1186570	453210	1106250	3147020	2560750
tr B3KPN7 B3KPN7_HUMAN;tr A0A024	4	0	304670	0	0	0	1018300	163690
tr E5RIM3 E5RIM3_HUMAN;sp Q9V263	4	0	298176	99780	2410900	237741	2796428	115940
tr A0A024R3Z2 A0A024R3Z2_HUMAN;s	4	0	296490	185223	611000	377200	1627580	878926
tr C9J0I7 C9J0I7_HUMAN;sp P35080-2	4	0	292670	0	469810	0	1863400	1372500
sp Q9Y3U8 RL36_HUMAN;tr J3QS85 J3	4	0	288890	248750	1060790	266780	58083000	5986020
sp Q99627-2 CSN8_HUMAN;tr E9PGT6	4	0	285860	451020	1022600	521360	19230000	352840
sp Q70CQ2-3 UBP34_HUMAN;sp Q70C	4	0.000362	284430	0	0	0	1649500	0
tr H3BTA2 H3BTA2_HUMAN;tr A0A024	4	0	279490	217020	589690	157830	3882400	510470
sp P49023-2 PAX1_HUMAN;tr F5GZ78	4	0	271640	164640	721940	280700	3162600	408370
sp Q95251-2 KAT7_HUMAN;sp Q95251-	4	0	267450	0	0	0	2285040	0
tr Q8IX26 Q8IX26_HUMAN;tr F5GZ13 F	4	0	262840	0	291950	0	3100700	0
tr F5H8F7 F5H8F7_HUMAN;tr B4DPT1	4	0	255560	0	0	0	4567200	0
tr V9HW51 V9HW51_HUMAN;tr B2R9C	4	0	250623	192080	703930	147590	7763000	512550
tr Q5TZN3 Q5TZN3_HUMAN;tr B2R4U3	4	0	237786	1810800	482090	287430	433450	215890
sp O15173 PGRC2_HUMAN;sp O15173-	4	0	237320	235020	640690	0	710600	0
sp Q9Y3D0 MIP18_HUMAN;tr H3BNV7	4	0	231760	205570	430410	109070	9184300	294280
tr F5H8D7 F5H8D7_HUMAN;tr A0A024	4	0	230040	0	445270	0	1043200	23293
sp Q8WWK9-6 CKAP2_HUMAN;tr B2R	4	0	229160	45278	1322100	0	3105948	0
tr A0A024RAS8 A0A024RAS8_HUMAN;	4	0	209970	1720800	2259520	2130300	5450100	1099700

tr B2RAR2 B2RAR2_HUMAN;sp Q9ULX	4	0	209430	0	330570	0	1971300	0
sp P80723 BASP1_HUMAN;sp P80723-	4	0	201420	89159	151090	0	0	0
sp Q95456-2 PSMG1_HUMAN;tr B2RD	4	0	184540	258950	763410	0	11054000	1231400
tr A0A024QZB1 A0A024QZB1_HUMAN;	4	0	183680	0	162740	0	4026760	0
sp Q9BW83-2 IFT27_HUMAN;sp Q9BW	4	0	175570	262350	0	0	3466800	502490
tr Q2LE71 Q2LE71_HUMAN;tr B2R4D5	4	0	159660	268720	806180	207090	8839500	395100
sp Q5JSZ5-5 PRC2B_HUMAN;sp Q5JSZ	4	0	155190	0	144190	0	1269100	0
tr B7WP74 B7WP74_HUMAN;sp Q9HC	4	0	142800	0	0	0	2299100	0
tr C9J2P0 C9J2P0_HUMAN;tr H7C061	4	0.00269	138040	147980	353180	0	1452100	0
tr A9QQ22 A9QQ22_HUMAN;tr A9QQ	4	0	136120	0	623900	74204	2046200	173340
sp Q9Y394-2 DHR57_HUMAN;sp Q9Y3	4	0	135750	155360	674430	93647	0	0
sp Q9BY32 ITPA_HUMAN;sp Q9BY32-2	4	0	128520	209680	829490	222510	6913800	348220
tr C9J384 C9J384_HUMAN;tr D3DN44	4	0	126700	0	292630	0	1620300	0
sp Q9Y5B6-2 PAXB1_HUMAN;sp Q9Y5	4	0.00037	123230	0	0	0	2897550	35671
tr Q6FHJ5 Q6FHJ5_HUMAN;sp O14828	4	0	119460	146300	664630	0	937160	0
sp Q75935-3 DCTN3_HUMAN;sp Q759	4	0	118410	0	0	0	2616000	810000
sp Q15051 IQCB1_HUMAN;sp Q15051-	4	0	100110	0	0	0	1571400	0
tr D3DR37 D3DR37_HUMAN;sp Q53EZ	4	0	95773	0	785160	0	963990	0
sp Q71UM5 RS27L_HUMAN;tr H0YMV4	4	0.000703	93193	147610	1220800	0	3187700	456320
tr Q96DB5-2 RMD1_HUMAN;sp Q96D	4	0	71838	0	315830	0	2404600	126690
tr B4E312 B4E312_HUMAN;sp Q92804-	4	0.005197	70581	0	211333	0	0	0
sp Q9NZL9-4 MAT2B_HUMAN;tr A8K7	4	0	58158	0	0	0	565280	856640
sp O00469 PLOC2_HUMAN;sp O00469	4	0	0	0	520080	0	0	0
sp O14727-3 APAF_HUMAN;tr A5YM4	4	0	0	0	0	0	1436300	0
sp O15260-2 SURF4_HUMAN;tr Q5T8U	4	0	0	0	0	0	4188750	0
sp O75419-2 CDC45_HUMAN;sp O7541	4	0	0	0	0	0	7965000	0
sp P35270 SPRE_HUMAN;tr Q9UEC5 C	4	0	0	0	321370	0	1741100	0
sp P38432 COIL_HUMAN;tr I3L369 I3L	4	0	0	0	541400	0	3419700	0
sp P41223 BUD31_HUMAN;sp P41223-	4	0	0	0	0	0	1884600	0
sp P42695 CNDD3_HUMAN;tr Q96FA6	4	0	0	0	0	0	2693700	0
sp P54687-4 BCAT1_HUMAN;tr A0A02	4	0	0	0	295640	0	509240	0
sp P61964 WDR5_HUMAN	4	0	0	0	0	0	1335810	0
sp Q00403 TF2B_HUMAN;tr B1APE1 B	4	0	0	0	323340	0	1871400	0
sp Q3ZCW2 LEGL_HUMAN;tr U3KQ88	4	0	0	0	0	0	1363210	0
sp Q5SY16 NOL9_HUMAN	4	0	0	0	0	0	1699000	0
sp Q6ZU65 UBN2_HUMAN;sp Q6ZU65-	4	0	0	0	121890	0	436530	0
sp Q7Z7H5-3 TMED4_HUMAN;sp Q7Z7	4	0	0	0	0	0	4425900	0
sp Q86V48-2 LUZP1_HUMAN;sp Q86V	4	0	0	0	0	0	1383600	0
sp Q86X76-2 NIT1_HUMAN;tr B7Z410	4	0	0	0	0	0	1143200	0
sp Q8IUF8-4 MINA_HUMAN;sp Q8IUF	4	0	0	0	407150	0	1628200	0
sp Q8WX93-7 PALLD_HUMAN;sp Q8W	4	0	0	0	1666980	0	2581600	0
sp Q8WZA9 IRGQ_HUMAN;tr B7ZMD6	4	0	0	0	427070	0	0	0
sp Q92889 XPF_HUMAN;tr A0PJAJ AC	4	0	0	0	486570	0	1888100	0
sp Q96GM5-2 SMRD1_HUMAN;sp Q96	4	0	0	0	259360	0	1089300	0
sp Q96JB1-2 DYH8_HUMAN;sp Q96JB1	4	0.000358	0	0	0	0	2753200	0
sp Q96P16-3 RPR1A_HUMAN;tr A0A02	4	0.000368	0	0	0	0	579700	0
sp Q96Q15-4 SMG1_HUMAN;sp Q96Q	4	0.000354	0	0	106560	0	1017300	0
sp Q9BWT3-2 PAPOG_HUMAN;sp Q9E	4	0.005393	0	0	0	0	472480	0
sp Q9BYJ9 YTHD1_HUMAN;tr B4DT65	4	0.000371	0	0	553900	0	2017400	0
sp Q9H6T3-3 RPAP3_HUMAN;sp Q9H6	4	0	0	0	0	0	1766200	0
sp Q9H8S9 MOB1A_HUMAN;sp Q7L9L	4	0	0	0	0	0	332840	0
sp Q9NUQ6-2 SPS2L_HUMAN;tr A0A02	4	0	0	0	1066290	0	6335200	0
sp Q9NVE7 PANK4_HUMAN;tr B4DHW	4	0	0	0	0	0	2901700	0
sp Q9UL40-3 ZN346_HUMAN;sp Q9UL	4	0	0	0	0	0	1359600	0
sp Q9ULK4-2 MED23_HUMAN;sp Q9UI	4	0	0	0	290170	0	1739400	0
sp Q9Y6W5 WASF2_HUMAN;tr Q05BU	4	0	0	0	0	0	4947400	0
tr A0A024QZH6 A0A024QZH6_HUMAN	4	0	0	0	0	0	1691700	0
tr A0A024RB31 A0A024RB31_HUMAN;s	4	0.000369	0	0	0	0	1678600	0
tr A0A087VV25 A0A087VV25_HUMAN	4	0	0	0	778090	0	8625700	0
tr A0A090N7W4 A0A090N7W4_HUMAN	4	0	0	0	0	0	1457600	0
tr A6QKW0 A6QKW0_HUMAN;tr A0AC	4	0	0	0	0	0	1329200	0
tr A8MTY9 A8MTY9_HUMAN;sp O1497	4	0	0	0	0	0	787690	0
tr B2RDJ6 B2RDJ6_HUMAN;sp O76071	4	0	0	0	0	0	1972000	0
tr B3KNC3 B3KNC3_HUMAN;sp Q9Y3T	4	0	0	0	0	0	1165490	0
tr B3KPC1 B3KPC1_HUMAN;tr A0A024	4	0	0	0	638370	0	1314200	0
tr B3KS28 B3KS28_HUMAN;sp Q147X3	4	0	0	0	0	0	1455300	0
tr B3KT11 B3KT11_HUMAN;sp Q96D46	4	0	0	0	0	0	3628400	0
tr B3KW08 B3KW08_HUMAN;sp Q96Q	4	0	0	0	400010	0	2834800	0
tr B4DFV1 B4DFV1_HUMAN;tr A0A024	4	0	0	0	0	0	3597500	0
tr B4DNU0 B4DNU0_HUMAN;tr Q53HI	4	0	0	0	281040	0	2961700	0
tr B4DW97 B4DW97_HUMAN;tr A0A08	4	0.000355	0	0	0	0	2085400	0
tr B4DZ22 B4DZ22_HUMAN;sp P28288	4	0	0	0	355200	0	0	0
tr B4E086 B4E086_HUMAN;tr K7EJH0	4	0	0	0	0	0	2650000	0
tr B4E253 B4E253_HUMAN;tr F5H5V6	4	0	0	0	132730	0	1111700	0
tr B7Z2V7 B7Z2V7_HUMAN;tr B7Z1V5	4	0	0	0	302830	0	4865400	0
tr B7ZA10 B7ZA10_HUMAN;tr B4E273	4	0	0	0	0	0	610950	0
tr C9J177 C9J177_HUMAN;sp Q15435-	4	0	0	0	0	0	0	0
tr C9JRI5 C9JRI5_HUMAN;sp Q9UGP4	4	0	0	0	0	0	4628400	0

tr E7EPJ7 E7EPJ7_HUMAN;sp O75962-1	4	0	0	0	0	0	0	1858100	0
tr E7ERH2 E7ERH2_HUMAN;tr F8W8N3	4	0	0	0	0	0	0	2013040	0
tr E9PCN5 E9PCN5_HUMAN;sp Q9H9A1	4	0	0	0	0	1049100	0	2955790	0
tr HOYEF3 HOYEF3_HUMAN;sp Q8TDP1	4	0	0	0	0	0	0	1657400	0
tr J3QR55 J3QR55_HUMAN	4	1	0	0	0	0	0	353810	0
tr MOR3B2 MOR3B2_HUMAN;tr A8K671	4	0	0	0	0	1082800	0	7110800	0
tr Q5TB52 Q5TB52_HUMAN;sp O95340	4	0	0	0	0	769920	0	0	0
tr Q63HP7 Q63HP7_HUMAN;tr Q5G0E1	4	0	0	0	0	550950	0	2372100	0
tr Q86TY2 Q86TY2_HUMAN;tr V9GYX7	4	0	0	0	0	343710	0	1929000	0
tr Q9BS45 Q9BS45_HUMAN;tr A0A024	4	0	0	0	0	466970	0	4906400	0
tr Q9UK43 Q9UK43_HUMAN;tr Q95HA1	4	0	0	0	0	397300	0	2189600	0
tr X6RAL5 X6RAL5_HUMAN;tr H7BZW6	4	0	0	0	0	0	0	2555500	0
tr A0A024R1F9 A0A024R1F9_HUMAN;sp	4	0	0	0	0	0	0	2925540	162790
sp Q9BWJ5 SF3B5_HUMAN	4	0	0	0	0	29406	0	2030000	140680
tr N0E4C7 N0E4C7_HUMAN;sp P67870	4	0	0	0	0	208320	0	3301000	235210
tr B5BUI8 B5BUI8_HUMAN;sp P51452	4	0	0	0	0	197150	0	4647100	332320
tr Q8IYS3 Q8IYS3_HUMAN;tr Q53FW7	4	0	0	0	0	0	0	3214800	253740
tr F5GYK2 F5GYK2_HUMAN;tr B4DMU1	4	0	0	0	0	191770	0	2103180	178050
tr Q05BU6 Q05BU6_HUMAN;tr Q6PIY5	4	0	0	0	0	1593600	0	18821920	1758169
sp P61962 DCAF7_HUMAN;tr B4DH46	4	0	0	0	0	0	0	5030800	470940
sp P46020-3 KPBI1_HUMAN;tr A6NIT2	4	0	0	0	0	0	0	2654460	286390
tr A0A024R7G6 A0A024R7G6_HUMAN;tr	4	0	0	0	0	0	0	1416100	203330
tr B4DRU9 B4DRU9_HUMAN;sp Q9NU11	4	0	0	0	0	0	0	1610400	274410
tr Q6IAV3 Q6IAV3_HUMAN;sp P41567	4	0	0	0	0	0	0	4838300	947610
sp O75340-2 PDCD6_HUMAN;tr Q53FC1	4	0	0	0	0	0	0	2183400	698180
tr Q53H22 Q53H22_HUMAN;tr A8K4H1	4	0	0	0	0	1788743	0	1014100	386570
sp Q7L5D6 GET4_HUMAN;sp Q7L5D6-2	4	0	0	0	0	147280	0	1272500	542270
tr Q6FGX9 Q6FGX9_HUMAN;tr Q53EY8	4	0	0	0	0	0	0	512210	377520
tr S4R3N1 S4R3N1_HUMAN;tr A8K012	4	0	0	0	0	422740	0	522410	514580
sp Q9Y237 PIN4_HUMAN;sp Q9Y237-2	4	0	0	0	0	109270	0	268790	421630
sp Q9BT22-8 DHRS4_HUMAN;sp Q9BT11	4	0	0	1146800	172380	0	0	622500	0
tr A2I829 A2I829_HUMAN;tr Q6NUN2	4	0	0	148100	0	0	0	2815840	0
tr B4DKV5 B4DKV5_HUMAN;sp Q96EK1	4	0	0	114540	169750	0	0	1550600	0
tr B7Z5N6 B7Z5N6_HUMAN;sp Q5T1V1	4	0	0	150580	350370	0	0	4412200	0
tr R4GND1 R4GND1_HUMAN;tr A0A024	4	0	0	148430	337530	0	0	7203400	160340
sp O75607 NPM3_HUMAN	4	0	0	337960	384290	0	0	20681000	891750
sp O94888 UBXN7_HUMAN;tr C9JAT7	4	0	0	274120	908427	0	0	2790600	531882
tr V9HWC9 V9HWC9_HUMAN;sp P00411	4	0	0	577800	0	0	0	0	1535910
sp Q9Y547 IFT25_HUMAN;tr A6NIR2 A	4	0	0	259920	588160	74696	0	10500000	658740
tr C9JG97 C9JG97_HUMAN;tr A0A024R	4	0	0	161580	1292500	316400	0	3006700	0
tr A8K6I6 A8K6I6_HUMAN;sp Q9NZ52	4	0	0	166750	820650	231640	0	2595400	494990
sp P62995-3 TRA2B_HUMAN;tr Q8N1H1	4	0	0	0	654870	218106	0	1836820	820301
sp Q53GG5-2 PDLI3_HUMAN;sp Q53GI1	4	0	0	269990	319110	119630	0	10410000	1233007
tr B5MCS9 B5MCS9_HUMAN;tr A4D101	4	0	0	455860	639460	246300	0	18602000	4082500
sp Q9NQ44 EXOS5_HUMAN;tr M0R050	4	0	0	252390	314760	148790	0	11199000	928210
tr B2R5I8 B2R5I8_HUMAN;sp Q13637	4	0	0	0	288660	141670	0	0	0
sp Q9UJ70 NAGK_HUMAN;sp Q9UJ70-1	4	0	0	0	0	44573	0	2003200	109330
tr H3BRV9 H3BRV9_HUMAN;tr A0A024R	4	0	0	0	0	65972	0	0	1042000
tr Q7Z612 Q7Z612_HUMAN;tr A0A024R	3	0	29833000	3508000	60867000	5914700	148340000	15003000	
tr S6AWD6 S6AWD6_HUMAN	3	0	23449010	33362540	30749068	27900360	70141900	48302050	
sp POCG22 DR4L1_HUMAN	3	1	15799860	0	0	0	0	0	0
tr B4DKW4 B4DKW4_HUMAN;sp Q8TE11	3	0.000359	13666300	9663400	9288400	11306300	11449600	1760370	
tr Q0EFA5 Q0EFA5_HUMAN;tr A8K9C1	3	0	7045000	6024300	4903160	6783900	4310280	3520070	
sp Q9Y3D6 FIS1_HUMAN	3	0	2427000	990480	6179600	590630	4575300	830970	
sp Q53RT3 APRV1_HUMAN	3	0	2338740	594350	845034	0	4547900	603259	
tr A0A024R5H0 A0A024R5H0_HUMAN;tr	3	0	2182610	790290	3726800	302200	20794020	1656200	
tr B3KRY3 B3KRY3_HUMAN;tr A0A024R	3	0	2121228	0	486920	0	0	167680	
tr A0A024R6D4 A0A024R6D4_HUMAN;tr	3	0	1973400	1172062	4735800	1285111	21572250	10383941	
tr B4DK32 B4DK32_HUMAN;sp Q14139	3	0	1817200	92459	609760	0	0	0	0
sp Q15738 NSDHL_HUMAN;tr C9JDR0	3	0	1796500	0	0	0	0	0	0
sp P63172 DYL1_HUMAN;tr Q5VTU3	3	0	1738900	344750	2190800	0	7032000	664550	
sp P62318-2 SMD3_HUMAN;sp P62318	3	0	1599300	2003610	4654560	1596420	30821000	9581875	
tr B4E240 B4E240_HUMAN;sp Q92575	3	0	1418200	0	839790	0	0	0	0
tr Q6FGG2 Q6FGG2_HUMAN;sp Q15831	3	0	1397400	441740	1870500	167680	3697500	689830	
tr Q4G1A8 Q4G1A8_HUMAN;sp Q13551	3	0	1313800	0	1673800	0	1555400	0	0
sp Q86X10-3 RLGPB_HUMAN;sp Q86X10	3	0	1201300	0	0	48685	2513300	0	0
tr B4DHS3 B4DHS3_HUMAN;tr Q59G45	3	0	1158120	0	1336400	0	4482700	0	0
tr C9JP00 C9JP00_HUMAN;sp Q9NR56	3	0	1080100	0	1781459	0	7173463	583020	
sp Q32MZ4-3 LRRF1_HUMAN;sp Q32MZ4	3	0	1053300	67582	210080	0	0	87870	
tr B7ZKN7 B7ZKN7_HUMAN;tr B7Z2X2	3	0	1024600	0	15727921	224490	294230	0	0
tr R4SBI6 R4SBI6_HUMAN;tr Q6FGZ3 Q	3	0	1002100	387540	0	0	0	0	0
tr Q59HA2 Q59HA2_HUMAN;tr Q6IBL8	3	0	946590	342700	1815200	171560	7354300	322870	
tr Q53RZ9 Q53RZ9_HUMAN;sp Q9HCN1	3	0	937070	0	2683700	0	0	897620	
tr B2RE34 B2RE34_HUMAN;tr A0A024R	3	0	906100	247530	1001800	151120	2301100	0	
sp O00461 GOLI4_HUMAN;tr F8W785	3	0	879030	434910	960260	241160	1708600	196880	



tr I3L2R3 I3L2R3_HUMAN;tr I3L533 I3L533_HUMAN;	3	0	771520	96498	1483200	360430	977160	204760
tr Q96J85 Q96J85_HUMAN;sp Q71RC2 Q71RC2_HUMAN;	3	0	760984	0	972290	0	2890330	0
tr A0A024QZC1 A0A024QZC1_HUMAN;	3	0	744000	0	0	0	2694700	230810
sp Q8WZAO-2 LZIC_HUMAN;tr A0A024QZC1 A0A024QZC1_HUMAN;	3	0	727560	0	533770	164990	0	331887
sp Q96DT7-3 ZBT10_HUMAN;tr A8E4L4 A8E4L4_HUMAN;	3	0	696960	0	166750	0	1295300	0
sp O94874-2 UFL1_HUMAN;sp O94874-2 UFL1_HUMAN;	3	0	693890	0	0	0	0	32691
sp Q5VVQ6-2 OTU1_HUMAN;sp Q5VVQ6-2 OTU1_HUMAN;	3	0	659440	0	0	0	6256000	0
sp P42684-7 ABL2_HUMAN;sp P42684-7 ABL2_HUMAN;	3	0	655150	323960	692100	250230	4297800	970950
tr A6NML8 A6NML8_HUMAN;sp O608 O608_HUMAN;	3	0	650070	0	410700	0	1676000	0
tr X6R9L0 X6R9L0_HUMAN;tr A8KA82 A8KA82_HUMAN;	3	0	638650	0	885540	0	3371800	0
sp Q4KMP7 TB10B_HUMAN	3	0	633340	162400	0	0	0	0
tr B4DNM0 B4DNM0_HUMAN;sp Q8WZAO-2 LZIC_HUMAN;	3	0	624410	0	380740	0	5799600	0
tr B4DQJ4 B4DQJ4_HUMAN;sp O15381 O15381_HUMAN;	3	0	611260	0	549590	0	2798300	0
sp Q9UPP1-4 PHF8_HUMAN;tr HOY3N9 HOY3N9_HUMAN;	3	0	594220	165430	232920	118930	1499100	0
sp Q9BRA2 TXD17_HUMAN;tr I3L0K2 I3L0K2_HUMAN;	3	0	593680	449770	1358300	0	9488600	1848500
sp Q9Y6H1 CHCH2_HUMAN;sp Q5T1J5 Q5T1J5_HUMAN;	3	0	570350	1470700	1419100	565500	663590	0
tr E5RHD8 E5RHD8_HUMAN;sp Q0539 Q0539_HUMAN;	3	0	563140	0	0	0	4026500	0
tr B4DG55 B4DG55_HUMAN;tr L7R50 L7R50_HUMAN;	3	0	545920	0	0	0	4953800	0
tr A0A024QYV8 A0A024QYV8_HUMAN	3	0	509440	0	312960	0	460930	0
sp Q8ND04 SMG8_HUMAN;sp Q8ND04 Q8ND04_HUMAN;	3	0.000373	499820	0	0	0	2983640	0
sp O75420 PERQ1_HUMAN;tr A2VDH9 A2VDH9_HUMAN;	3	0	492680	135540	215180	0	1690200	0
sp Q13131 AAPK1_HUMAN;sp Q13131 Q13131_HUMAN;	3	0.00273	489090	0	0	0	0	0
tr A0A024R1S5 A0A024R1S5_HUMAN;s	3	0	487470	0	0	0	1581800	0
tr C9J8T0 C9J8T0_HUMAN;sp P57772 P57772_HUMAN;	3	0	469620	0	963680	0	1552290	0
tr B2R9T9 B2R9T9_HUMAN;sp Q9BVC6 Q9BVC6_HUMAN;	3	0	454520	1301100	681674	0	100780	637880
sp Q6YP21-3 KAT3_HUMAN;tr B4DW1 B4DW1_HUMAN;	3	0	452330	0	703790	0	2355200	0
tr B7Z3S8 B7Z3S8_HUMAN;sp Q8N122 Q8N122_HUMAN;	3	0	451480	0	0	0	2846600	0
sp Q03164-2 KMT2A_HUMAN;sp Q03164-2 KMT2A_HUMAN;	3	0	448060	0	0	0	0	0
tr A0A024R7W0 A0A024R7W0_HUMAN	3	0	444480	0	2497800	194230	595890	0
tr A0A024RDA1 A0A024RDA1_HUMAN	3	0	435760	272736	542590	0	2490240	0
sp P11171-6 41_HUMAN;sp P11171-4 P11171-4_HUMAN;	3	0.000363	420570	0	0	0	472770	0
sp Q9HBK9-2 AS3MT_HUMAN;tr A0A0 A0A0_HUMAN;	3	0	399060	0	1279500	0	1993800	227580
tr A0A024R6N2 A0A024R6N2_HUMAN;	3	0	397120	161077	0	0	1253700	0
sp Q4J6C6-4 PPCEL_HUMAN;sp Q4J6C6-4 Q4J6C6-4_HUMAN;	3	0	391370	0	0	0	0	0
tr HOYE29 HOYE29_HUMAN;sp Q07960 Q07960_HUMAN;	3	0	387810	0	1205400	0	0	0
tr Q6FGH9 Q6FGH9_HUMAN;sp P6316 P6316_HUMAN;	3	0	384240	386101	1231300	664030	4028800	1902020
sp Q96RU2-2 UBP28_HUMAN;sp Q96RU2-2 UBP28_HUMAN;	3	0.000356	383310	0	228460	0	0	0
sp Q15542 TAF5_HUMAN;sp Q15542-2 Q15542-2_HUMAN;	3	0	371900	0	0	0	3092400	0
sp Q7Z589-2 EMSY_HUMAN;sp Q7Z589-2 Q7Z589-2_HUMAN;	3	0	362690	0	59819	0	980810	0
tr B7ZMB3 B7ZMB3_HUMAN;sp Q5TC8 Q5TC8_HUMAN;	3	0.000369	355700	0	0	0	510300	0
tr E7ER32 E7ER32_HUMAN;tr A4FUJ8 A4FUJ8_HUMAN;	3	0	353340	0	651620	0	708610	0
sp P60468 SC61B_HUMAN;tr Q53FA5 Q53FA5_HUMAN;	3	0.000703	347110	0	795680	167850	0	825480
tr C9JP85 C9JP85_HUMAN;sp Q16656 Q16656_HUMAN;	3	0.002699	341430	0	680320	0	1112900	0
sp Q69YN4-2 VIR_HUMAN;sp Q69YN4-2 VIR_HUMAN;	3	0	336000	0	0	0	4341900	0
tr HOY8L0 HOY8L0_HUMAN	3	1	329960	0	0	0	0	0
tr B7Z361 B7Z361_HUMAN;tr A0A024R7W0 A0A024R7W0_HUMAN;	3	0	318390	415660	963150	293030	1694600	327920
tr A8K7B1 A8K7B1_HUMAN;sp Q96T51 Q96T51_HUMAN;	3	0.000367	301920	0	237830	0	566800	0
tr A0A024R094 A0A024R094_HUMAN;t	3	0	301870	0	501130	0	1766900	188140
tr A0A0A0MRX2 A0A0A0MRX2_HUMAN	3	0	298640	0	993990	0	0	0
sp Q6BDS2 URFB1_HUMAN;tr H7C1J4 H7C1J4_HUMAN;	3	0	292530	226140	412170	0	1146700	0
tr H1UBN3 H1UBN3_HUMAN;tr Q5U0M Q5U0M_HUMAN;	3	0	288280	0	587070	0	685420	0
tr B4E2V7 B4E2V7_HUMAN;tr A0A087XOM4 A0A087XOM4_HUMAN;	3	0	285430	0	144200	0	1525900	0
tr A0A024RBR4 A0A024RBR4_HUMAN;	3	0	283780	0	354790	0	552910	0
tr A0A024R2G8 A0A024R2G8_HUMAN;	3	0	282510	0	213400	0	873290	0
tr J3QRD1 J3QRD1_HUMAN;sp P51648 P51648_HUMAN;	3	0	279020	0	0	0	0	35626
sp Q96JH7 VCIP1_HUMAN;tr B4DM84 B4DM84_HUMAN;	3	0	268940	537350	380840	0	1499900	0
sp Q5BKZ1 ZN326_HUMAN	3	0	267400	0	0	0	3021800	0
tr Q6I9V5 Q6I9V5_HUMAN;tr A8K787 A8K787_HUMAN;	3	0.006571	262020	0	0	0	0	0
tr B2R841 B2R841_HUMAN;sp P53350 P53350_HUMAN;	3	0	257170	340130	835290	0	0	0
tr B4DV85 B4DV85_HUMAN;tr A0A024QZC1 A0A024QZC1_HUMAN;	3	0	250170	0	236750	0	1343500	0
sp P52630-4 STAT2_HUMAN;tr R9QGC R9QGC_HUMAN;	3	0	238780	0	0	0	2377900	0
tr A0A087XOM4 A0A087XOM4_HUMAN	3	0	231670	0	0	0	0	71342
tr Q2NLC8 Q2NLC8_HUMAN;tr E9PFN5 E9PFN5_HUMAN;	3	0	229741	0	0	106943	576400	36082
sp Q9NVH2-4 INT7_HUMAN;sp Q9NVH2-4 Q9NVH2-4_HUMAN;	3	0	223810	111750	301520	54940	712960	0
tr A0A024R1D6 A0A024R1D6_HUMAN;	3	0	221520	0	862280	93961	2104500	0
sp Q969U7-2 PSMG2_HUMAN;tr K7EN K7EN_HUMAN;	3	0	215890	0	546030	0	5627900	57276
sp P48507 GSHO_HUMAN;sp P48507-2 P48507-2_HUMAN;	3	0	213110	181270	704470	0	1086500	328170
sp Q9BTY7 HGH1_HUMAN;tr Q9POTS Q9POTS_HUMAN;	3	0.00332	202810	0	502470	0	0	0
tr B4DEZ8 B4DEZ8_HUMAN;tr B2RCG9 B2RCG9_HUMAN;	3	0	202510	0	175810	0	1782100	75029
tr D6RB55 D6RB55_HUMAN;sp Q8I281 Q8I281_HUMAN;	3	0.000358	200037	0	259230	0	0	0
sp Q14686 NCOA6_HUMAN;tr F6M2K3 F6M2K3_HUMAN;	3	0	174620	0	208800	0	3190300	73459
tr F5GXX5 F5GXX5_HUMAN;sp P61803 P61803_HUMAN;	3	0	171930	0	277260	0	0	0
sp POCJ79 ZN888_HUMAN	3	0	164580	0	0	0	1463090	0
sp O14893-2 GEMI2_HUMAN;sp O14893-2 GEMI2_HUMAN;	3	0	162170	318140	579330	142690	2066300	138930
sp O95248 MTMR5_HUMAN;tr G5E933 G5E933_HUMAN;	3	0	156600	0	358010	0	1634300	371690

sp Q7KZ17-10 MARK2_HUMAN;tr A0A0	3	0	155230	0	633610	0	399620	0
tr B2R4R9 B2R4R9_HUMAN;sp P62857	3	0	154250	55076	251360	0	1366400	6820955
tr Q53XM7 Q53XM7_HUMAN;sp O952	3	0	149650	126380	1103200	180130	578020	0
sp O75170-6 PP6R2_HUMAN;sp O7517	3	0	146860	0	0	0	568050	0
sp Q86W92 LIPB1_HUMAN;tr F5GZP6	3	0	142730	0	0	0	738820	0
tr A8CDT9 A8CDT9_HUMAN;tr A0A087	3	0.002055	142240	0	0	0	422600	0
tr B7Z6B3 B7Z6B3_HUMAN;tr B7Z2A3	3	0	137860	79791	123090	0	393390	301850
tr V9HW53 V9HW53_HUMAN;sp O958	3	0	132330	191690	281770	0	1049700	0
tr Q6IN90 Q6IN90_HUMAN;tr E5RUV1	3	1	126810	0	0	0	0	0
tr I3L3Q7 I3L3Q7_HUMAN;tr I3L3B0 I3	3	0.00037	124190	1093700	0	0	0	0
tr G8IU5 G8IU5_HUMAN;tr A0A024QY	3	0	116790	0	138700	0	545910	0
sp Q9H7E9 CH033_HUMAN;sp Q9H7E9	3	0	115310	123540	375430	78725	668040	146100
sp Q96H20-2 SNF8_HUMAN;sp Q96H2	3	0	112560	539260	1342630	432560	7114100	677500
tr D3DWK4 D3DWK4_HUMAN;tr B4DG	3	0	110360	0	247520	0	587770	0
tr Q549M9 Q549M9_HUMAN;sp P5637	3	0	107900	152270	813560	143270	8690600	171880
tr A0A024R1T1 A0A024R1T1_HUMAN;s	3	0	104610	0	382510	0	4111200	0
sp P60983 GMFB_HUMAN;tr G3V4P8	3	0	98777	155890	0	0	8746900	0
sp Q9UL5-3 PRR12_HUMAN;sp Q9UL5	3	0.00037	94693	0	0	0	908860	0
tr Q49AG2 Q49AG2_HUMAN;tr B4DDR	3	0	91011	0	145460	0	3270500	186410
tr V9HW91 V9HW91_HUMAN;sp Q9N	3	0	90003	152550	198970	125720	0	430130
tr A8K7A1 A8K7A1_HUMAN;sp Q9Y6V	3	0.0007	84111	0	692770	0	2224100	0
tr B4DHX2 B4DHX2_HUMAN;tr A0A024	3	0	79610	0	47899	0	1371700	0
sp Q9Y3Y2-4 CHTOP_HUMAN;tr X6R7C	3	0	51565	0	150170	0	9797500	53213
tr E7EQ72 E7EQ72_HUMAN;tr B4DP27	3	0	47365	0	92038	78019	2735800	0
tr A8K724 A8K724_HUMAN;tr A0A024	3	0	43945	0	401430	0	1736200	0
sp Q0VF96 CGNL1_HUMAN;tr Q6P5Q2	3	0.000355	24815	379782	2328519	256087	148039	1460671
sp A6NKF9 GPHRC_HUMAN;sp B7ZAQ	3	0.000365	0	0	0	0	0	0
sp O60293-2 ZC3H1_HUMAN;sp O6029	3	0	0	0	0	0	1495200	0
sp O60645-2 EXOC3_HUMAN;tr Q69YF	3	0	0	0	588860	0	1397000	0
sp O76041 NEBL_HUMAN;tr F6TRT2 F	3	0.003316	0	0	0	0	139270	0
sp O76075-2 DFFB_HUMAN;tr A0A024	3	0	0	0	0	0	1935800	0
sp O95425-2 SVIL_HUMAN;sp O95425	3	0	0	0	0	0	445870	0
sp P04818-2 TYSY_HUMAN;tr Q53Y97	3	0	0	0	0	0	0	0
sp P07305 H10_HUMAN;sp P07305-2	3	0	0	0	0	0	1795600	0
sp P07954-2 FUMH_HUMAN;sp P07954	3	0	0	0	0	0	0	0
sp P25325 THTM_HUMAN;sp P25325-2	3	0	0	0	0	0	2806800	0
sp P41214 EIF2D_HUMAN;tr Q5SY38 C	3	0	0	0	0	0	3242700	0
sp P46013-2 KI67_HUMAN;tr A0A087V	3	0.000703	0	0	0	0	1190300	0
sp P46109 CRKL_HUMAN	3	0	0	0	0	0	0	0
sp P46734-2 MP2K3_HUMAN;tr C7DUV	3	0	0	0	0	0	965070	0
sp P48729 KC1A_HUMAN;tr V9HW00	3	0	0	0	0	0	532810	0
sp P53680-2 AP2S1_HUMAN;sp P5368	3	0.000697	0	0	654940	0	3171500	0
sp P54198-2 HIRA_HUMAN;sp P54198	3	0	0	0	0	0	1260300	0
sp P55145 MANF_HUMAN;tr A8K878	3	0	0	0	0	0	0	0
sp P55210-4 CASP7_HUMAN;sp P5521	3	0.000361	0	0	280180	0	2418500	0
sp P62487 RPB7_HUMAN;tr E9PIU7 E9	3	0	0	0	170810	0	988430	0
sp P78524 ST5_HUMAN;tr E9PPL2 E9P	3	0.000368	0	0	0	0	0	0
sp Q05682-5 CALD1_HUMAN;tr E9PGZ	3	0	0	0	0	0	0	0
sp Q13445 TMED1_HUMAN;tr K7EIN4	3	0.00037	0	0	0	0	1164600	0
sp Q15139 KPCD1_HUMAN;tr Q1KKQ2	3	0	0	0	282660	0	475420	0
sp Q5C924 NOM1_HUMAN	3	0	0	0	0	0	1468000	0
sp Q5ISL3 DOC11_HUMAN;tr A6NIW2	3	0	0	0	0	0	1744300	0
sp Q5JTH9-2 RRP12_HUMAN;sp Q5JTH	3	0	0	0	0	0	971180	0
sp Q6IA86-4 ELP2_HUMAN;sp Q6IA86	3	0.000702	0	0	0	0	0	0
sp Q6P2H3-2 CEP85_HUMAN;sp Q6P2	3	0	0	0	0	0	2672400	0
sp Q86U38-2 NOP9_HUMAN;tr Q5HYL	3	0	0	0	0	0	1086600	0
sp Q86VS8 HOOK3_HUMAN;tr H0YE69	3	0	0	0	0	0	1070200	0
sp Q8ND24-2 RN214_HUMAN;tr A0A0	3	0	0	0	337400	0	2781600	0
sp Q8WXEO-2 CSK12_HUMAN;sp Q8W	3	0	0	0	0	0	2164800	0
sp Q93100-4 KPBB_HUMAN;sp Q93100	3	0	0	0	0	0	2762800	0
sp Q96BD5-2 PF21A_HUMAN;sp Q96B	3	0	0	0	1726500	0	2651900	0
sp Q96GX9 MTNB_HUMAN;tr B4DY17	3	0	0	0	0	0	29719	0
sp Q96KQ7-2 EHMT2_HUMAN;tr A0AC	3	0.000372	0	0	0	0	669490	0
sp Q9BXS6-7 NUSAP_HUMAN;sp Q9B	3	0	0	0	0	0	1303500	0
sp Q9BYG3 MK671_HUMAN;tr B4DSM4	3	0	0	0	0	0	622420	0
sp Q9H0A8-2 COMD4_HUMAN;tr H3B	3	0.001375	0	0	0	0	1051600	0
sp Q9H0L4 CSTFT_HUMAN	3	0.005392	0	0	0	0	1558700	0
sp Q9H840 GEMI7_HUMAN	3	0	0	0	0	0	4362300	0
sp Q9HBM1 SPC25_HUMAN;tr C9IW94	3	0	0	0	50755	0	1023200	0
sp Q9NRY4 RHG35_HUMAN;tr A2RRE5	3	0	0	0	0	0	1746700	0
sp Q9NUD5 ZCHC3_HUMAN;sp Q9NUI	3	0	0	0	0	0	2065300	0
sp Q9NW08-2 RPC2_HUMAN;tr Q7Z3F	3	0	0	0	0	0	1885900	0
sp Q9NW64-2 RBM22_HUMAN;sp Q9N	3	0	0	0	0	0	3233200	0
sp Q9P031 TAP26_HUMAN;tr F8VNY5	3	0	0	0	0	0	938050	0
sp Q9UBS4 DJB11_HUMAN;tr B3KW63	3	0	0	0	477820	0	0	0
sp Q9UEG4 ZN629_HUMAN;sp Q8N8L	3	0	0	0	0	0	1186680	0

sp Q9UNP9-2 PPIE_HUMAN;tr A8KAM	3	0	0	0	168440	0	2036200	0
sp Q9Y2L5-2 TPPC8_HUMAN;tr J3QQU5	3	0	0	0	0	0	746490	0
sp Q9Y3P9 RBGP1_HUMAN;sp Q9Y3P9	3	0	0	0	317510	0	2278500	0
sp Q9Y608-2 LRRF2_HUMAN;sp Q9Y608	3	0	0	0	0	0	1129400	0
tr A0A024QYR8 A0A024QYR8_HUMAN;	3	0	0	0	617320	0	0	0
tr A0A024QZE9 A0A024QZE9_HUMAN;	3	0	0	0	0	0	2629900	0
tr A0A024ROV9 A0A024ROV9_HUMAN;	3	0.000362	0	0	180600	0	1155800	0
tr A0A024R1U2 A0A024R1U2_HUMAN;	3	0	0	0	0	0	1885300	0
tr A0A024R4M8 A0A024R4M8_HUMAN	3	0	0	0	34921	0	1456100	0
tr A0A024R8K8 A0A024R8K8_HUMAN;s	3	0	0	0	0	0	1982300	0
tr A0A024R9K4 A0A024R9K4_HUMAN;s	3	0	0	0	0	0	3041100	0
tr A0A024R9R3 A0A024R9R3_HUMAN;s	3	0	0	0	0	0	1452600	0
tr A0A024RD28 A0A024RD28_HUMAN;	3	0	0	0	0	0	1731800	0
tr A0A024RE27 A0A024RE27_HUMAN;s	3	0	0	0	0	0	4638100	0
tr A0A0A0MR02 A0A0A0MR02_HUMAN	3	0.000353	0	0	201560	0	0	0
tr A0PJK4 A0PJK4_HUMAN;tr B3KTT0	3	0	0	0	0	0	5040440	0
tr A4UCU2 A4UCU2_HUMAN	3	0	0	0	905250	0	1151200	0
tr A8K2F9 A8K2F9_HUMAN;sp Q9BWH	3	0.000357	0	0	294350	0	860880	0
tr A8K330 A8K330_HUMAN;sp Q8IWA	3	0	0	0	387731	0	0	0
tr A8K4A1 A8K4A1_HUMAN;sp Q96HV	3	0	0	0	228390	0	1548500	0
tr B1AJQ6 B1AJQ6_HUMAN;tr Q6LEU0	3	0.005208	0	0	985850	0	0	0
tr B2R6H6 B2R6H6_HUMAN;sp Q9HBM	3	0	0	0	0	0	2243300	0
tr B3KM65 B3KM65_HUMAN;tr Q59H2	3	0	0	0	1467890	0	907300	0
tr B4DH39 B4DH39_HUMAN;tr Q8TAF6	3	0.000356	0	0	1534100	0	945690	0
tr B4DSN8 B4DSN8_HUMAN	3	0	0	0	0	0	738510	0
tr B4DUJ0 B4DUJ0_HUMAN;tr Q5QPM	3	0	0	0	0	0	1481200	0
tr B4DX46 B4DX46_HUMAN;sp Q6GMV	3	0	0	0	677410	0	4156000	0
tr B4DZF6 B4DZF6_HUMAN;tr Q59F94	3	0.003327	0	0	0	0	756470	0
tr B5BUF9 B5BUF9_HUMAN;tr B5BU32	3	0	0	0	155540	0	3077400	0
tr B5BUK7 B5BUK7_HUMAN;tr B2RCZ4	3	0	0	0	0	0	1565490	0
tr B7SBB1 B7SBB1_HUMAN;tr G9I2H4	3	0	0	0	514690	0	586150	0
tr B9A6K1 B9A6K1_HUMAN;tr A0A024	3	0	0	0	0	0	3022490	0
tr C9IJ54 C9IJ54_HUMAN;tr Q9H8N9 C	3	0	0	0	671290	0	1504000	0
tr C9JXC3 C9JXC3_HUMAN;tr C9JNE2	3	0	0	0	0	0	1252600	0
tr E5RIS3 E5RIS3_HUMAN;sp Q8TCF1-4	3	0.005405	0	0	0	0	1019900	0
tr E7EW84 E7EW84_HUMAN;tr B4DEZ1	3	0	0	0	0	0	1635500	0
tr F5H442 F5H442_HUMAN;sp Q99816	3	0.000371	0	0	659600	0	2004900	0
tr H0YI09 H0YI09_HUMAN;tr A0A024R	3	0	0	0	188560	0	0	0
tr I6L969 I6L969_HUMAN;sp Q8TES7-5	3	0.000364	0	0	487400	0	106300	0
tr J3KS22 J3KS22_HUMAN;tr J3QS36 J	3	0	0	0	0	0	429020	0
tr Q5U523 Q5U523_HUMAN;sp Q9UL4	3	0.000359	0	0	812340	0	4110890	0
tr Q643R0 Q643R0_HUMAN;sp Q9ULV	3	0.000692	0	0	150520	0	0	0
tr Q69YG1 Q69YG1_HUMAN;sp P58546	3	0	0	0	0	0	0	0
tr Q6FG85 Q6FG85_HUMAN;tr Q53F41	3	0.005998	0	0	0	0	0	0
tr Q6IBK3 Q6IBK3_HUMAN;tr A8K769	3	0	0	0	193090	0	214500	0
tr Q6ICQ8 Q6ICQ8_HUMAN;sp P84095	3	0	0	0	0	0	1476000	0
tr Q8N523 Q8N523_HUMAN;tr A0A024	3	0	0	0	342100	0	3808400	0
sp Q9NY12-2 GAR1_HUMAN;tr A0A024	3	0	0	0	0	0	5238700	149960
tr H7C5L6 H7C5L6_HUMAN;tr A0A024F	3	0.0052	0	0	0	0	2551800	74204
sp O75494-5 SR510_HUMAN;tr Q5JRI1	3	0	0	0	255340	0	5252240	166630
tr Q7Z6U0 Q7Z6U0_HUMAN;tr Q7Z6U1	3	0	0	0	143690	0	1765400	75554
tr K7ENX8 K7ENX8_HUMAN;tr K7ELV6	3	0	0	0	241030	0	4868400	238280
tr Q5SYZ4 Q5SYZ4_HUMAN;sp P29373	3	0	0	0	0	0	3224000	171690
tr B8ZZS4 B8ZZS4_HUMAN;tr A0A024F	3	0	0	0	0	0	2222400	129830
sp Q9UQ88-5 CD11A_HUMAN;sp Q9U	3	0	0	0	0	0	1842900	122610
tr B2R7W3 B2R7W3_HUMAN;sp O7593	3	0	0	0	416080	0	3658500	249670
tr Q9NUF9 Q9NUF9_HUMAN;sp Q1322	3	0	0	0	234170	0	1499100	184310
sp Q16890-4 TPD53_HUMAN;sp Q1689	3	0	0	0	0	0	848230	107520
tr A8K646 A8K646_HUMAN;sp Q92882	3	0	0	0	0	0	7557770	980170
tr Q5QPP3 Q5QPP3_HUMAN;tr Q5QPP	3	0	0	0	835310	0	1539300	320590
tr A9CQZ4 A9CQZ4_HUMAN	3	0.006606	0	0	0	0	1865200	397772
sp Q96T60-2 PNKP_HUMAN;sp Q96T6	3	0	0	0	235110	0	394890	98399
sp Q9BTE6 AASD1_HUMAN;tr C9J5N1	3	0.000362	0	0	0	0	2387700	603370
sp O15530-4 PDPK1_HUMAN;tr E9PER	3	0.004274	0	0	142420	0	682720	205960
sp Q9BYB4 GNB1L_HUMAN;sp Q9BYB4	3	0.000356	0	0	0	0	1051600	341330
sp Q9NP97 DLR81_HUMAN;tr B4DFR2	3	0	0	0	252760	0	4413900	1488300
sp Q9Y333 LSM2_HUMAN	3	0	0	0	0	0	3254500	2228700
tr E9PNC7 E9PNC7_HUMAN;sp Q1491	3	0	0	0	0	0	424660	858050
sp A6ZKI3 F127A_HUMAN;sp Q9BWD3	3	0.000364	0	0	176610	0	0	353660
tr Q8WVC2 Q8WVC2_HUMAN;tr Q6FC	3	0	0	0	146650	0	0	3184100
sp O14757-2 CHK1_HUMAN;tr J3KN87	3	0	0	32129	632380	0	428380	0
sp O60921-2 HUS1_HUMAN;tr A4D2F2	3	0	0	65703	159510	0	1784700	0
sp Q7Z6E9-2 RBBP6_HUMAN;sp Q7Z6E	3	0	0	264387	388130	0	3007000	0
sp Q9Y4Z0 LSM4_HUMAN;tr V9GZ56 V	3	0	0	151430	593493	0	4067400	0
tr A0A024R688 A0A024R688_HUMAN;s	3	0	0	1375000	2741700	0	5553800	0



tr B7Z7C0 B7Z7C0_HUMAN;sp Q16537	3	0	0	138000	0	0	525460	0
tr C9JUT4 C9JUT4_HUMAN;tr B7ZLW0	3	0	0	83534	0	0	2893100	0
sp Q96AT9-2 RPE_HUMAN;sp Q96AT9	3	0	0	602950	0	0	15506000	304380
sp Q9BXK1 KLF16_HUMAN;tr D6W5Z8	3	0	0	93849	112570	0	1830600	67313
sp Q8NCW5-2 NNRE_HUMAN;sp Q8N	3	0	0	167830	317600	0	2257500	747520
sp Q8IUR0 TPPC5_HUMAN;tr MQQZQ8	3	0.000703	0	0	1565400	112390	0	0
sp Q9H8Y8-2 GORS2_HUMAN;tr B4DEI	3	0	0	0	1397400	149070	1107300	373360
tr B9EGR5 B9EGR5_HUMAN;sp Q8IWI9	3	0	0	0	302890	58108	568130	0
sp Q9UFW8 CGBP1_HUMAN;tr C9JUI0	3	0	0	0	955870	186150	1190300	312450
tr B3KQC8 B3KQC8_HUMAN;tr Q4G10	3	0	0	0	1239210	274660	0	0
sp Q9Y3B4 SF3B6_HUMAN	3	0	0	0	519130	137350	5394600	603690
tr F8VP97 F8VP97_HUMAN;sp Q9NZN	3	0	0	0	1692100	453070	499680	878080
tr B3KQ71 B3KQ71_HUMAN;tr B4DWL	3	0	0	228430	634610	186040	1132500	0
tr B4EQQ6 B4EQQ6_HUMAN;tr B4DKZ7	3	0	0	134010	413470	128080	2256700	145440
tr E7EQL8 E7EQL8_HUMAN;tr A7E2V7	3	0	0	0	298360	117780	2628200	0
sp Q8TBA6-2 GOGA5_HUMAN;tr A8KA	3	0	0	0	1586800	645125	925850	854320
tr Q7Z508 Q7Z508_HUMAN;tr A0A087	3	0	0	206280	243720	163840	0	218780
sp P62273 RS29_HUMAN;tr A0A087W	3	0	0	0	134120	113070	6022300	397920
tr Q53GC2 Q53GC2_HUMAN;tr A0A02	3	0	0	155120	181800	158000	133330	0
tr A0A087WYR0 A0A087WYR0_HUMAN	3	0	0	0	0	49668	2548500	89493
sp Q15843 NEDD8_HUMAN;tr F8VSA6	3	0	0	0	0	318930	2480340	3414600
sp Q8IW75 SPA12_HUMAN	3	0.000693	0	376196	0	559972	0	0
tr B3KPA1 B3KPA1_HUMAN;sp O1465	3	0	0	629770	0	253100	1316600	0
tr H3BRS3 H3BRS3_HUMAN;tr B4DXS2	3	0	0	70828	0	125390	0	0
sp P62304 RUXE_HUMAN;tr A6NHK2	3	0	0	674550	0	473530	23667000	6852600
sp Q5T750 XP32_HUMAN	3	0.002673	0	437100	0	437736	864110	714270
sp P11532-3 DMD_HUMAN;sp P11532	2	0	134101000	44785000	5656600	81339000	20305000	18501500
tr Q6FHL9 Q6FHL9_HUMAN;tr B1AKZ5	2	0.001378	9865900	6263350	0	7759600	0	8933400
sp P80188-2 NGAL_HUMAN;sp P80188	2	0	7523200	0	0	0	1152500	0
tr K7EMV3 K7EMV3_HUMAN;tr K7EP0	2	0	6185830	2635313	8098370	271780	20307620	2373230
sp Q66G59 CP135_HUMAN	2	0.000693	3312290	0	0	0	715290	0
sp Q9GZZ8 LACRT_HUMAN;tr F8W0V3	2	0	2371500	90026	765060	1469120	0	300069
tr A0A024R326 A0A024R326_HUMAN;s	2	0	2255430	988780	5313800	825810	39899620	2717740
sp O95969 SG1D2_HUMAN	2	0	2080950	1120220	1131440	1485740	1558110	1921040
sp P35813-2 PPM1A_HUMAN;tr B5BUI	2	0	1933400	532350	1744900	490840	2641860	810310
sp P62877 RBX1_HUMAN	2	0	1686500	2103410	4663800	1233900	15210000	1926300
sp P25815 S100P_HUMAN	2	0	1651983	174466	198225	159767	300560	168501
tr B4DMY4 B4DMY4_HUMAN;tr B4DL4	2	0.000367	1571640	0	0	0	0	0
sp P08047-2 SP1_HUMAN;sp P08047 S	2	0	1536200	644960	402040	697870	8418800	0
tr G3V1B8 G3V1B8_HUMAN;tr H0YEB6	2	0.000354	1516500	174390	190020	0	1135000	116840
tr Q76LA1 Q76LA1_HUMAN;sp P04080	2	0	1472347	0	161250	0	1116200	3191600
tr F5H3C5 F5H3C5_HUMAN;sp P04179	2	0	1371900	0	0	0	0	0
tr B4E2S7 B4E2S7_HUMAN;sp P13473-	2	0	1332110	471340	0	348960	280040	159082
tr M0R132 M0R132_HUMAN;tr W8GIV	2	0.000354	1313660	0	0	0	0	0
sp P04062-4 GLCM_HUMAN;tr A0A068	2	0	1212000	0	0	0	0	0
tr A8K6H9 A8K6H9_HUMAN;tr A0A024	2	0	1197020	229348	0	334719	0	0
tr K7E1Y6 K7E1Y6_HUMAN;tr A8K0Q1 A	2	0.000693	1172600	147910	2061690	0	1354741	0
tr F5H0V4 F5H0V4_HUMAN;sp Q9HAP	2	0	1162200	0	476490	0	0	0
tr C9JYN0 C9JYN0_HUMAN;sp Q16563	2	0	969020	0	0	0	217150	101390
tr Q5T085 Q5T085_HUMAN;tr Q6NSB3	2	0	929010	712930	0	621190	0	927120
tr B2RAR3 B2RAR3_HUMAN;sp Q9BXR	2	0	925700	0	0	0	1356000	939230
sp Q16610-2 ECM1_HUMAN;sp Q1661	2	0	896010	542800	453260	620230	545730	0
tr A0A024R4V8 A0A024R4V8_HUMAN;	2	0	891470	0	607620	0	2918450	0
tr Q5JVD1 Q5JVD1_HUMAN;sp Q7Z7A	2	0	861430	0	0	0	0	0
tr Q6FG99 Q6FG99_HUMAN	2	0.000372	850350	516210	2255000	1151200	14686290	0
tr A0A024R8T9 A0A024R8T9_HUMAN;s	2	0.000363	830290	0	748220	0	1720700	341150
sp Q8NBX0 SCPDL_HUMAN	2	0.000372	805790	320590	499710	0	0	0
sp P35326 SPR2A_HUMAN;sp P35325	2	0	785150	571140	872110	770496	2140140	2364390
sp Q96A33 CCD47_HUMAN;tr A0A087	2	0	779880	0	1569800	0	0	0
sp Q6GTS8 P20D1_HUMAN	2	0.002689	716540	0	0	0	0	0
tr A0A024R277 A0A024R277_HUMAN;s	2	0	703180	0	110470	0	0	0
tr H0YH81 H0YH81_HUMAN;tr Q0QEN	2	0	695680	0	1676500	207960	0	0
tr B3KM89 B3KM89_HUMAN;tr A8K6V	2	0	659020	0	202355	0	1269700	0
tr E7EWM1 E7EWM1_HUMAN;sp Q155	2	0	646530	142760	277640	0	983910	0
tr Q5H9S0 Q5H9S0_HUMAN;tr B4DTN6	2	0	641810	0	0	0	769430	0
tr B4DVB5 B4DVB5_HUMAN;tr A0A02	2	0	631190	0	1080400	235450	2912200	0
tr Q05BV7 Q05BV7_HUMAN;tr B4DYZ	2	0	626010	0	0	0	528060	0
tr A0A024R8D7 A0A024R8D7_HUMAN;	2	0	621620	672940	0	994520	634470	1024650
tr Q59G54 Q59G54_HUMAN;sp Q9NRL	2	0	610190	0	0	0	0	0

tr G1UCX3 G1UCX3_HUMAN;sp Q96ER	2	0	598420	0	714100	0	1441100	0
tr Q5SYF9 Q5SYF9_HUMAN;sp Q7Z4H7	2	0	596470	79153	489260	0	750080	0
tr E5RIF2 E5RIF2_HUMAN;tr Q59FY5 C	2	0.003624	562840	0	350900	0	0	0
sp Q9NS87-2 KIF15_HUMAN;sp Q9NS8	2	0	549740	0	0	0	0	0
tr Q5HY57 Q5HY57_HUMAN;sp P50402	2	0	543900	104420	634210	0	555000	0
tr B4DZG6 B4DZG6_HUMAN;sp Q98TX	2	0.000359	508360	0	0	0	0	0
tr E9PP67 E9PP67_HUMAN;tr E9PQ25	2	0.000364	471290	0	0	0	0	0
sp Q49A26-5 GLYR1_HUMAN;sp Q49A	2	0	471000	67537	0	0	393470	0
sp O75592-2 MYCB2_HUMAN;sp O755	2	0.004271	469270	0	0	0	820550	0
sp P01622 KV304_HUMAN;tr G3GAU4	2	0	464860	0	125520	0	878270	0
tr A4D110 A4D110_HUMAN	2	0	463400	1041100	20768000	7426700	0	0
tr H7C1T3 H7C1T3_HUMAN;tr D3DXB2	2	0.0027	459930	0	0	193180	3236000	787020
sp P41229-4 KDM5C_HUMAN;tr A6NG6	2	0	456360	0	234950	0	0	0
sp Q96PY6-4 NEK1_HUMAN;sp Q96PY	2	0.001373	452130	0	159340	0	253150	0
tr Q05CT8 Q05CT8_HUMAN;sp Q8NHV	2	0	445000	146910	708250	178500	2361000	272190
tr B2RAN2 B2RAN2_HUMAN;sp Q9545	2	0.002999	424555	0	15800	0	0	0
tr A8KAK5 A8KAK5_HUMAN;tr O6024	2	0.005418	412550	0	0	0	0	0
sp Q9ULU4-4 PKCB1_HUMAN;sp Q9UL	2	0	405010	0	0	0	1851800	0
tr E7ETU5 E7ETU5_HUMAN;tr F6YSH0	2	0	397580	0	0	0	2317400	0
tr C9J2I1 C9J2I1_HUMAN;tr G5E9V6 G	2	0	395530	0	0	0	1091700	0
sp P48163-2 MAOX_HUMAN;tr A8K16	2	0	394682	0	457080	153190	0	120920
tr B4DWZ7 B4DWZ7_HUMAN;tr B3KTM	2	0	388840	204550	483860	159030	1631300	247990
sp P62861 RS30_HUMAN;tr E9PR30 E9	2	0.006805	383780	0	0	142690	20927000	4413690
tr Q5T093 Q5T093_HUMAN;tr Q5T092	2	0	375200	0	246770	0	1453110	0
sp O00186 STXB3_HUMAN	2	0	368090	112630	747770	0	0	0
sp O43913-2 ORC5_HUMAN;tr Q9UDM	2	0	363290	0	1379400	0	1713900	0
sp Q9H4L7-3 SMRCD_HUMAN;sp Q9H4	2	0.005397	360900	0	0	0	0	57990
sp Q8N1G2 CMTR1_HUMAN	2	0	345800	0	0	0	0	0
sp O43156 TTI1_HUMAN;tr Q3B768 Q	2	0	343450	0	245980	0	1450600	0
sp Q9BRP4-3 PAAF1_HUMAN;tr A0A0	2	0	340320	0	420230	0	1077200	0
sp Q7Z4L5 TT21B_HUMAN;sp Q7Z4L5-	2	0.006604	334280	0	0	0	3470500	0
sp Q9UIQ6-3 LCAP_HUMAN;sp Q9UIQ	2	0.002674	329960	0	0	0	0	0
sp Q86WBO-3 NIPA_HUMAN;tr C9J0I9	2	0	328450	0	0	145140	1048800	0
tr S5DU05 S5DU05_HUMAN;tr F6IQK6	2	0	313640	227650	0	230690	0	0
tr B4DR88 B4DR88_HUMAN;sp Q8IX18	2	0	313020	0	464560	0	1642400	0
tr A0A024R8B1 A0A024R8B1_HUMAN;s	2	0.000692	307540	275970	0	0	0	0
sp Q96JG6-3 CC132_HUMAN;tr A7MD	2	0	296090	0	412700	0	1748800	0
tr B7Z4K5 B7Z4K5_HUMAN;tr A8MT72	2	0.003622	292350	84084	70360	0	1005900	23427
tr B4DY59 B4DY59_HUMAN;tr E9PF19	2	0	285480	0	554530	0	0	0
sp Q9H4L4 SEN3_HUMAN;tr J3QL36	2	0	282870	0	417580	0	1470200	0
tr Q15203 Q15203_HUMAN;tr Q7KZ52	2	0	274740	1257141	735750	653186	0	7909100
sp P33981-2 TTK_HUMAN;sp P33981	2	0	269030	0	0	0	1086300	0
sp Q9H9B1-4 EHMT1_HUMAN;sp Q9H9	2	0.000358	264210	0	233110	0	734760	0
tr A8K335 A8K335_HUMAN;sp Q92820	2	0.007367	224837	0	0	0	0	49220
tr A8K1V4 A8K1V4_HUMAN;sp Q1578	2	0.000362	199750	0	475980	0	457370	0
sp Q6P1Q9 MET2B_HUMAN;tr F8WAS	2	0.00426	197710	0	294990	0	566600	0
tr D3DSB5 D3DSB5_HUMAN;tr B2R928	2	0.002707	197650	0	0	0	0	0
tr A0A024QYW3 A0A024QYW3_HUMAN	2	0	196531	21552	1674800	263590	728800	0
tr HOYFD2 HOYFD2_HUMAN;tr F8WFC	2	0	181350	0	0	0	1018300	0
sp Q14669-4 TRIPC_HUMAN;sp Q1466	2	0	179500	0	170710	0	1381500	0
tr F8VUA7 F8VUA7_HUMAN;tr F8VQX	2	0.009691	173860	0	0	0	0	0
tr D3DR32 D3DR32_HUMAN;sp Q96Q8	2	0.005431	173310	0	142170	0	0	0
tr E9PRF4 E9PRF4_HUMAN;sp Q15047	2	0.003003	163630	0	0	0	849880	0
tr Q53HG5 Q53HG5_HUMAN;tr A8K4K	2	0	134110	0	448590	0	499480	0
sp Q9BVC4 LST8_HUMAN;tr H3BR38 H	2	0.002703	123290	0	0	0	1532700	0
tr E9PIY1 E9PIY1_HUMAN;tr B4DUI2 B	2	0.006776	118480	0	0	0	0	0
sp Q8IXW5 RPAP2_HUMAN;sp Q8IXW	2	0	114830	0	0	0	387220	0
tr D3VVC3 D3VVC3_HUMAN	2	0.00652	112080	0	0	0	579040	0
tr HOY4F4 HOY4F4_HUMAN;tr F8W6D9	2	0.002727	109350	0	0	0	428640	0
sp Q9UQN3-2 CHM2B_HUMAN;tr A0A	2	0	101880	115910	460110	106620	465630	0
tr A0A024QYT6 A0A024QYT6_HUMAN;	2	0	100890	171030	320350	0	2809700	242680
sp Q15652-2 JHD2C_HUMAN;tr B7ZLC	2	0	100780	0	0	0	765990	0
sp Q9NP72 RAB18_HUMAN;tr Q59EW	2	0.000372	91285	281790	153600	0	669170	0
tr A6NGW1 A6NGW1_HUMAN;tr A8K5	2	0	90459	0	173230	0	848730	0
sp Q9H6V9 CB043_HUMAN;sp Q9H6V	2	0	90361	97669	161210	0	655590	0
tr A0A024QYX0 A0A024QYX0_HUMAN;	2	0	90032	0	139220	0	0	0
sp Q9BT22 ALG1_HUMAN;tr K7EID2 K	2	0	85190	0	0	0	0	0
tr F5H1S8 F5H1S8_HUMAN;tr F5GX14	2	0	84776	0	234590	0	345910	0
sp Q96HR3-2 MED30_HUMAN;sp Q96H	2	0	79812	0	216630	0	1624540	0
sp Q9BXB4 OSB11_HUMAN;tr Q9GZM	2	0.006591	55082	0	0	0	0	0
tr Q6ZS01 Q6ZS01_HUMAN;sp O60281	2	1	45252	0	1966100	0	0	0
tr C9JDZ2 C9JDZ2_HUMAN;sp Q9NVQ	2	0	44138	0	60425	0	2264870	0
sp A6NDU8 CE051_HUMAN	2	0	0	0	0	0	1831800	0
sp O14646-2 CHD1_HUMAN;sp O1464	2	0.001375	0	0	0	0	1033200	0
sp O15116 LSM1_HUMAN	2	0	0	0	0	0	809990	0

sp O43301 HS12A_HUMAN;tr B7Z2M8	2	0.004255	0	0	0	0	532710	0
sp O43414-3 ERI3_HUMAN;tr B4DEX5	2	0.0007	0	0	0	0	2835700	0
sp O43474-4 KLF4_HUMAN;sp O43474	2	0	0	0	368820	0	3207700	0
sp O75182-2 SIN3B_HUMAN;sp O7518	2	0	0	0	56249	0	636800	0
sp O75191-2 XYLB_HUMAN;sp O75191	2	0	0	0	286400	0	137280	0
sp O95721 SNP29_HUMAN;tr C9JAF7	2	0	0	0	282490	0	485950	0
sp P05412 JUN_HUMAN;tr Q6FHK0 Q	2	0	0	0	2355400	0	4888800	0
sp P10746 HEM4_HUMAN;tr Q5T3L7 C	2	0.005202	0	0	0	0	1906000	0
sp P21359-2 NF1_HUMAN;sp P21359-6	2	0.000365	0	0	0	0	926430	0
sp P55201-4 BRPF1_HUMAN;sp P5520	2	0.00517	0	0	0	0	1050200	0
sp P58004 SES2_HUMAN	2	0.000361	0	0	341590	0	1420500	0
sp Q02040-2 AK17A_HUMAN;sp Q0204	2	0	0	0	0	0	1544900	0
sp Q12974-3 TP4A2_HUMAN;sp Q12974	2	0	0	0	538260	0	1319159	0
sp Q13427-2 PPIG_HUMAN;tr Q6GMS5	2	0	0	0	0	0	259820	0
sp Q15475 SIX1_HUMAN;sp Q9NPC8 S	2	0	0	0	0	0	1195300	0
sp Q1ED39 KNOP1_HUMAN;tr H3BNU	2	0	0	0	0	0	2825200	0
sp Q3B726 RPA43_HUMAN	2	0.003629	0	0	0	0	1246300	0
sp Q3KQU3-2 MA7D1_HUMAN;sp Q3KQ	2	0.005444	0	0	0	0	752780	0
sp Q5JTV8-3 TOIP1_HUMAN;tr J3KN66	2	0	0	0	697480	0	0	0
sp Q5T0B9 ZN362_HUMAN;tr F5H055	2	0.002736	0	0	0	0	82820	0
sp Q5THR3-5 EFCB6_HUMAN;sp Q5TH	2	0.005387	0	0	3601140	0	0	0
sp Q6IBW4-2 CNDH2_HUMAN;sp Q6IE	2	0	0	0	493810	0	0	0
sp Q6P582 MZT2A_HUMAN;sp Q6NZ6	2	0.000366	0	0	0	0	1541000	0
sp Q6PK04 CC137_HUMAN;tr I3LOU5 I	2	0	0	0	0	0	686810	0
sp Q6SPF0 SAMD1_HUMAN;tr E9PIW5	2	0.002706	0	0	0	0	230010	0
sp Q71F23-2 CENPU_HUMAN;tr Q09GI	2	0.00736	0	0	0	0	130910	0
sp Q7Z4Q2 HEAT3_HUMAN;sp Q7Z4Q	2	0.000368	0	0	0	0	2600600	0
sp Q8IWV7 UBR1_HUMAN;sp Q8IWV7	2	0.009949	0	0	0	0	567590	0
sp Q8N6M0 OTU6B_HUMAN;tr A0A08	2	0	0	0	0	0	1556200	0
sp Q8N954 GPT11_HUMAN;tr A0A0A0	2	0.003627	0	0	0	0	700950	0
sp Q8NAV1 PR38A_HUMAN;tr B2RDP	2	0	0	0	574460	0	2460400	0
sp Q8NEY1-7 NAV1_HUMAN;sp Q8NE	2	0.001374	0	0	0	0	848700	0
sp Q8TEU7-5 RPGF6_HUMAN;sp Q8TE	2	0.000353	0	0	0	0	601850	0
sp Q8WW12-2 PCNP_HUMAN;sp Q8W	2	0	0	0	0	0	395930	0
sp Q92520 FAM3C_HUMAN;tr C9JP35	2	0.000364	0	0	146755	0	884340	0
sp Q92558 WASF1_HUMAN;tr Q55ZK4	2	0.009685	0	0	475330	0	1247630	0
sp Q96MG7 MAGG1_HUMAN	2	0	0	0	0	0	270990	0
sp Q96MU7-2 YTD1_HUMAN;sp Q96M	2	0.003623	0	0	0	0	2254840	0
sp Q96MW1 CCD43_HUMAN;tr Q86W	2	0.006309	0	0	0	0	598930	0
sp Q96P11-5 SUN5_HUMAN;sp Q96P	2	0	0	0	0	0	1734800	0
sp Q96PV6 LENG8_HUMAN;tr A0A087	2	0.00425	0	0	0	0	1265900	0
sp Q99747-2 SNAG_HUMAN;tr Q6FHY	2	0.000704	0	0	0	0	1996100	0
sp Q9BU89 DOHH_HUMAN;tr K7ERU8	2	0	0	0	0	0	1423100	0
sp Q9GZR2 REXO4_HUMAN;tr B4E331	2	0.005445	0	0	0	0	1349100	0
sp Q9GZS1-2 RPA49_HUMAN;sp Q9GZ	2	0.000368	0	0	0	0	3965400	0
sp Q9GZT4 SRR_HUMAN;tr Q3ZK31 Q	2	0	0	0	0	0	1287400	0
sp Q9H759 ZN703_HUMAN;tr A0A0A0	2	0.000359	0	0	656670	0	307700	0
sp Q9H832-2 UBE2Z_HUMAN;tr B4DL6	2	0.000356	0	0	264770	0	2952500	0
sp Q9HCE5 MET14_HUMAN;tr B4DJF7	2	0	0	0	0	0	2280100	0
sp Q9HD40 SPCS_HUMAN;sp Q9HD40	2	0.003636	0	0	51008	0	0	0
sp Q9NQE9 HINT3_HUMAN	2	0.002731	0	0	0	0	287930	0
sp Q9NRH1 YAED1_HUMAN;tr C9IZ57	2	0	0	0	0	0	1294300	0
sp Q9NV56 MRGBP_HUMAN	2	0	0	0	111330	0	1298200	0
sp Q9NW82 WDR70_HUMAN;tr D6RIV	2	0	0	0	1021300	0	3012300	0
sp Q9NYJ8-2 TAB2_HUMAN;tr B4DIR9	2	0	0	0	335590	0	430070	0
sp Q9NZQ3-3 SPN90_HUMAN;sp Q9N	2	0.002717	0	0	1067200	0	0	0
sp Q9P2D3-3 HTR5B_HUMAN;tr B9EK4	2	0	0	0	0	0	1037400	0
sp Q9UHD2 TBK1_HUMAN;tr B4E164 I	2	0.005395	0	0	0	0	595870	0
sp Q9UIV1-2 CNOT7_HUMAN;tr Q96IC	2	0	0	0	258070	0	3445900	0
sp Q9UK99-3 FBX3_HUMAN;sp Q9UK9	2	0	0	0	0	0	748120	0
sp Q9UKB1-2 FBW1B_HUMAN;tr B4DH	2	0.000354	0	0	269750	0	0	0
sp Q9UKL0 RCOR1_HUMAN;tr J3KN32	2	0.000369	0	0	503950	0	1966920	0
sp Q9Y3A4 RRP7A_HUMAN	2	0	0	0	0	0	1889000	0
sp Q9Y6A4 CFA20_HUMAN;tr B4DN94	2	0.000367	0	0	60256	0	851570	0
sp Q9Y6X8 ZHX2_HUMAN	2	0.006794	0	0	0	0	1354680	0
tr A0A024R029 A0A024R029_HUMAN;s	2	0.007941	0	0	0	0	1071100	0
tr A0A024R5K1 A0A024R5K1_HUMAN;s	2	0.002696	0	0	608660	0	1301400	0
tr A0A024R611 A0A024R611_HUMAN;s	2	0.000363	0	0	148560	0	463250	0
tr A0A024R806 A0A024R806_HUMAN;s	2	0.000705	0	0	120170	0	0	0
tr A0A024R8G1 A0A024R8G1_HUMAN;	2	0.000361	0	0	0	0	1333600	0
tr A0A024RDS3 A0A024RDS3_HUMAN;	2	0.002712	0	0	0	0	968840	0
tr A0A087VYQ5 A0A087VYQ5_HUMAN;	2	0.003307	0	0	0	0	352250	0
tr A0A087X057 A0A087X057_HUMAN;t	2	0	0	0	152580	0	212800	0
tr A0A087X271 A0A087X271_HUMAN;t	2	0	0	0	0	0	419100	0
tr A0A090N7U2 A0A090N7U2_HUMAN	2	0	0	0	491010	0	867330	0

tr A0A0A0MQR4 A0A0A0MQR4_HUMA	2	0.00653	0	0	0	0	1280500	0
tr A0PJ71 A0PJ71_HUMAN;tr A8K1D3	2	0.000361	0	0	187590	0	1164150	0
tr A6PVP4 A6PVP4_HUMAN;tr B7ZBQ	2	0.00654	0	0	346092	0	1065200	0
tr A8K719 A8K719_HUMAN;sp Q13951	2	0	0	0	0	0	899340	0
tr A8K874 A8K874_HUMAN;sp Q5VZE5	2	0.006583	0	0	0	0	565570	0
tr B1AMY1 B1AMY1_HUMAN;tr B1AM	2	0.006792	0	0	0	0	1836400	0
tr B2RDG4 B2RDG4_HUMAN;sp Q1539	2	0.000697	0	0	0	0	541050	0
tr B2RUU3 B2RUU3_HUMAN;sp Q1418	2	0	0	0	287950	0	2432800	0
tr B3KNS8 B3KNS8_HUMAN;sp O7568	2	0	0	0	0	0	695680	0
tr B3KPC7 B3KPC7_HUMAN;tr A0A024	2	0.000357	0	0	228560	0	561970	0
tr B4DDR0 B4DDR0_HUMAN;sp Q9NV	2	0.000369	0	0	98142	0	579871	0
tr B4DE40 B4DE40_HUMAN;sp P52788	2	0	0	0	1111100	0	1293500	0
tr B4DFD7 B4DFD7_HUMAN;tr F5H658	2	0.005438	0	0	0	0	2037400	0
tr B4DFU6 B4DFU6_HUMAN;sp Q6IPR	2	0.000695	0	0	0	0	533600	0
tr B4DKR0 B4DKR0_HUMAN;sp Q1319	2	0	0	0	0	0	1694500	0
tr B4DLW5 B4DLW5_HUMAN;tr A0A09	2	0	0	0	0	0	2309900	0
tr B4DNS2 B4DNS2_HUMAN;tr Q5D1D	2	0.000701	0	0	0	0	1189700	0
tr B4DQ03 B4DQ03_HUMAN;tr A0A08	2	0.005183	0	0	98091	0	213590	0
tr B4DQX8 B4DQX8_HUMAN;sp Q9UK	2	0.00036	0	0	0	0	472430	0
tr B4DTZ4 B4DTZ4_HUMAN;tr A0A024	2	0	0	0	0	0	1137400	0
tr B4DZX4 B4DZX4_HUMAN	2	1	0	0	88403	0	0	0
tr B4E2T4 B4E2T4_HUMAN;tr E9PS42	2	0.002681	0	0	0	0	751680	0
tr B4E308 B4E308_HUMAN;tr B4DI83	2	0	0	0	0	0	1009900	0
tr B5BU41 B5BU41_HUMAN;tr B0YIY3	2	0	0	0	0	0	468730	0
tr B5MDQ0 B5MDQ0_HUMAN;sp Q2N	2	0	0	0	0	0	915000	0
tr B7Z3Z9 B7Z3Z9_HUMAN;tr E7EPM6	2	0.006796	0	0	0	0	1331200	0
tr B8ZZP4 B8ZZP4_HUMAN;tr C9J7U9	2	0.003946	0	0	512370	0	1044600	0
tr C9J3L8 C9J3L8_HUMAN;tr C9J5W0	2	0	0	0	1618500	0	484890	0
tr C9J979 C9J979_HUMAN;tr A0A024R	2	0.002725	0	0	0	0	863080	0
tr C9JB42 C9JB42_HUMAN;tr C9JDR7	2	0.007353	0	0	0	0	729090	0
tr C9JDF5 C9JDF5_HUMAN;sp Q9NP71	2	0	0	0	0	0	657760	0
tr C9JFV9 C9JFV9_HUMAN;sp O95639	2	0.006809	0	0	0	0	1597400	0
tr C9JVP0 C9JVP0_HUMAN;sp Q9UPW	2	0	0	0	0	0	851090	0
tr D3DQ54 D3DQ54_HUMAN;sp Q8N3	2	0.005383	0	0	0	0	1368800	0
tr D3VVJ8 D3VVJ8_HUMAN;tr G3V4U5	2	0.000353	0	0	88076	0	239930	0
tr D6R9R7 D6R9R7_HUMAN;tr B4DHK6	2	0	0	0	0	0	608920	0
tr D6RAD4 D6RAD4_HUMAN;sp P5061	2	0.005473	0	0	0	0	857290	0
tr D6W5D1 D6W5D1_HUMAN;sp Q3V6	2	0.004253	0	0	0	0	630480	0
tr E7EN32 E7EN32_HUMAN;tr A0A024	2	0	0	0	0	0	1806900	0
tr E7EVL6 E7EVL6_HUMAN;tr C9JZE3	2	0	0	0	0	0	725460	0
tr E9PDR5 E9PDR5_HUMAN;tr B7ZLD4	2	0.009099	0	0	0	0	513220	0
tr E9PKG6 E9PKG6_HUMAN;tr V9HW7	2	0.002714	0	0	0	0	491600	0
tr E9PKN0 E9PKN0_HUMAN;tr B7Z7X4	2	0	0	0	0	0	2067200	0
tr E9PL47 E9PL47_HUMAN;tr F8W860	2	0.005402	0	0	0	0	578410	0
tr E9PMI6 E9PMI6_HUMAN;tr E9PJF4	2	0	0	0	0	0	1348000	0
tr E9PMS6 E9PMS6_HUMAN;tr B7Z8W	2	0.007362	0	0	0	0	1132700	0
tr E9PPY3 E9PPY3_HUMAN;sp O43159	2	0	0	0	0	0	1140500	0
tr F5GZF0 F5GZF0_HUMAN;sp O14519	2	0.000365	0	0	0	0	145820	0
tr F5H0U5 F5H0U5_HUMAN;tr A0A024	2	0.000706	0	0	168550	0	19621600	0
tr F5H452 F5H452_HUMAN;tr Q5JSB6	2	0.002734	0	0	681140	0	2535600	0
tr F8VQR7 F8VQR7_HUMAN;tr A0A02	2	0.000363	0	0	0	0	763440	0
tr F8VYV2 F8VYV2_HUMAN;tr F8VNZ6	2	0.009976	0	0	0	0	57707	0
tr F8W0J4 F8W0J4_HUMAN;sp O95615	2	0.000363	0	0	0	0	348030	0
tr F8W8I6 F8W8I6_HUMAN;sp P31483	2	0	0	0	0	0	691120	0
tr F8WBV5 F8WBV5_HUMAN;tr G3XAJ	2	0.005215	0	0	111380	0	1890700	0
tr G3V195 G3V195_HUMAN;sp Q32P4	2	0.000358	0	0	0	0	511990	0
tr G3V2H7 G3V2H7_HUMAN;sp Q86SZ	2	0.00656	0	0	0	0	319040	0
tr G3V2N2 G3V2N2_HUMAN;sp Q9UUL	2	0	0	0	373280	0	1230400	0
tr HOY565 HOY565_HUMAN;tr D3DW0	2	0.006799	0	0	0	0	591190	0
tr HOY5S9 HOY5S9_HUMAN;tr HOY682	2	0.000355	0	0	0	0	1200000	0
tr HOY6B5 HOY6B5_HUMAN;tr Q53SH3	2	0	0	0	105060	0	1945300	0
tr H3BQ06 H3BQ06_HUMAN;sp Q9ULF	2	0.003628	0	0	0	0	604130	0
tr H3BRL9 H3BRL9_HUMAN;sp P78549	2	0	0	0	0	0	1721200	0
tr H3BS42 H3BS42_HUMAN;tr B2RD90	2	0.002695	0	0	0	0	12388800	0
tr H7C2A6 H7C2A6_HUMAN;tr C9JIK8	2	0.000357	0	0	402680	0	0	0
tr I3L252 I3L252_HUMAN;tr HOYCY6 H	2	0	0	0	0	0	928590	0
tr I3L2W9 I3L2W9_HUMAN;tr B4E2D6	2	0.000359	0	0	87743	0	1673270	0
tr J3QLW7 J3QLW7_HUMAN;tr B3KM4	2	0.001034	0	0	0	0	1554700	0
tr K7ERP4 K7ERP4_HUMAN;sp P36969	2	0.001715	0	0	0	0	405080	0
tr Q05DB8 Q05DB8_HUMAN;tr Q53F18	2	0.000369	0	0	0	0	995910	0
tr Q2NLD4 Q2NLD4_HUMAN;tr Q2NLC	2	0	0	0	0	0	911150	0
tr Q32MN6 Q32MN6_HUMAN;tr B4DP	2	0.002682	0	0	0	0	1344400	0
tr Q53G15 Q53G15_HUMAN;tr B7Z6D5	2	0.002716	0	0	0	0	2192200	0
tr Q53RX3 Q53RX3_HUMAN;sp Q9H8F	2	0	0	0	112100	0	912180	0
tr Q541A5 Q541A5_HUMAN;sp Q9289	2	0	0	0	0	0	1202300	0
tr Q59G93 Q59G93_HUMAN;tr J3KQ61	2	0.00036	0	0	115630	0	299600	0

tr Q59HE6 Q59HE6_HUMAN;tr A0A024	2	0	0	0	0	0	0	689550	0
tr Q51WM4 Q51WM4_HUMAN;sp Q9H	2	0.002722	0	0	0	0	0	2231800	0
tr Q5VVL2 Q5VVL2_HUMAN;tr B4DIY2	2	0.000372	0	0	0	0	0	392730	0
tr Q6FI27 Q6FI27_HUMAN;tr B5BUC0	2	0.006516	0	0	682980	0	0	589150	0
tr Q6GMQ7 Q6GMQ7_HUMAN;tr Q5JL	2	0.001378	0	0	0	0	0	1062900	0
tr Q6I9T1 Q6I9T1_HUMAN;tr Q68DB7	2	0	0	0	420810	0	0	1010500	0
tr Q6P9C2 Q6P9C2_HUMAN;tr B2RAL9	2	0	0	0	0	0	0	1513600	0
tr Q8N8L9 Q8N8L9_HUMAN;tr Q6PIW	2	0.000692	0	0	0	0	0	324500	0
tr Q96KB7 Q96KB7_HUMAN;sp Q9ULV	2	0.000701	0	0	440310	0	0	0	0
tr Q9NXD3 Q9NXD3_HUMAN;tr H0Y6L	2	0.005193	0	0	0	0	0	859130	0
tr Q9Y5Q4 Q9Y5Q4_HUMAN;sp A8MM	2	0.000697	0	0	179620	0	0	0	0
tr R4GMU7 R4GMU7_HUMAN;sp Q6D	2	0	0	0	106200	0	0	2288300	0
tr R4GN54 R4GN54_HUMAN;tr B3KY73	2	0.000358	0	0	0	0	0	2166800	0
tr S4R3K7 S4R3K7_HUMAN;tr S4R469	2	0.002683	0	0	661050	0	0	2047200	0
tr A0A024R6Q2 A0A024R6Q2_HUMAN;	2	0	0	0	0	0	0	2246700	151150
sp P24390-2 ERD21_HUMAN;sp P2439	2	0	0	0	0	0	0	2868500	240000
tr H3BV23 H3BV23_HUMAN;tr H3BUV	2	0.00037	0	0	0	0	0	2731800	308030
tr H3BPK3 H3BPK3_HUMAN;tr H3BPQ	2	0.005477	0	0	90950	0	0	821250	95720
tr Q75M98 Q75M98_HUMAN;tr Q75M	2	0	0	0	343440	0	0	2568600	302060
sp Q9NRA8-2 4ET_HUMAN;tr A0A024F	2	0	0	0	129410	0	0	1042900	126060
tr Q49AN9 Q49AN9_HUMAN;tr F5H01	2	0	0	0	0	0	0	2803200	387400
sp Q6PCE3 PGM2L_HUMAN	2	0.005459	0	0	0	0	0	877540	152470
tr E9PQR7 E9PQR7_HUMAN;tr Q548N	2	0.000366	0	0	0	0	0	1124700	199100
sp Q6UB99 ANR11_HUMAN;tr Q9UHR	2	0.002693	0	0	0	0	0	460810	83493
sp Q96CP2 FWCH2_HUMAN;tr I3L1Y9	2	0.001374	0	0	0	0	0	387900	71631
sp Q9NXF7 DCA16_HUMAN	2	0.002708	0	0	0	0	0	1475500	349100
tr H7C218 H7C218_HUMAN;tr H7C0X5	2	0.002396	0	0	0	0	0	849080	255450
tr Q6I6B8 Q6I6B8_HUMAN;sp Q99417	2	0	0	0	183510	0	0	520900	182450
tr B2R7U5 B2R7U5_HUMAN;tr A0A024	2	0.003322	0	0	0	0	0	283230	128370
sp O95295 SNAPN_HUMAN	2	0	0	0	239130	0	0	543300	437820
sp O15511 ARPC5_HUMAN;sp O15511	2	0	0	0	0	0	0	235470	781870
sp Q9H0F7 ARL6_HUMAN;sp Q9H0F7-	2	0.003001	0	0	160950	0	0	0	158350
tr R4GMS9 R4GMS9_HUMAN;tr Q86U	2	0.000368	0	0	311710	0	0	0	290658
sp P48059 LIMS1_HUMAN;sp P48059-	2	0	0	207080	0	0	0	7366500	0
sp P63218 GBG5_HUMAN	2	0	0	83713	165190	0	0	2798620	0
sp Q7L4I2-2 RSRC2_HUMAN;sp Q7L4I2	2	0	0	182630	144830	0	0	0	0
sp Q9BQ69 MACD1_HUMAN	2	0	0	150410	0	0	0	2607400	0
tr B3KNA1 B3KNA1_HUMAN;tr B3KT7	2	0	0	844404	0	0	0	977630	0
tr B4DDC2 B4DDC2_HUMAN;sp P3232	2	0	0	155840	278650	0	0	922390	0
tr G3V2Y4 G3V2Y4_HUMAN;tr H0YI20	2	0	0	150110	239690	0	0	0	0
tr H7C0G7 H7C0G7_HUMAN;sp Q9H9C	2	0	0	155590	0	0	0	1142600	0
tr U3KQU7 U3KQU7_HUMAN;sp Q9UP	2	0.009952	0	164310	389430	0	0	0	0
tr E9PRR7 E9PRR7_HUMAN;sp Q14331	2	0	0	108120	207460	0	0	3113600	183300
sp Q5HYI8 RABL3_HUMAN;tr H7C533	2	0	0	136680	0	0	0	4083100	294380
sp Q8YI6 EXOC8_HUMAN	2	0	0	106380	453100	0	0	1919000	232120
sp Q8W242-5 TITIN_HUMAN;tr C0JYZ2	2	0.004261	0	263420	0	0	0	1036500	694810
sp Q8IZP0-2 ABI1_HUMAN;sp Q8IZP0-	2	0	0	186450	1001400	209140	0	3180600	345920
tr A0A024RBV5 A0A024RBV5_HUMAN;	2	0	0	277320	697630	163730	0	0	0
sp Q9Y4X5 ARI1_HUMAN;tr H3BSK4 H	2	0	0	567390	1305500	331060	0	794120	0
sp Q6I49-2 SDE2_HUMAN;sp Q6I49	2	0.000702	0	0	565560	170000	0	1182600	319100
sp P33908 MA1A1_HUMAN;sp P33908	2	0.000366	0	180190	575310	182130	0	0	0
tr D3DSR2 D3DSR2_HUMAN;sp P0542	2	0	0	0	449120	154850	0	1037000	0
sp Q9BU76-4 MMTA2_HUMAN;sp Q9E	2	0	0	98469	133690	46501	0	735350	143013
sp P61956-2 SUMO2_HUMAN;tr A0A0	2	0	0	2684220	7379060	3018060	31712280	6472810	0
tr A8K818 A8K818_HUMAN;sp O1544	2	0	0	321330	664860	283620	5219000	0	0
sp Q9HC07 TM165_HUMAN;tr V9GYC8	2	0	0	335120	1409110	654560	0	0	0
tr A6NKE1 A6NKE1_HUMAN;sp O4361	2	0.002717	0	203490	170120	133840	1125600	321000	0
sp Q9UBI6 GBG12_HUMAN;tr Q53GD1	2	0.000367	0	109600	264400	275860	310720	0	0
tr Q9BX72 Q9BX72_HUMAN	2	1	0	1923720	1324900	2046160	4755070	4650800	0
tr Q69YR1 Q69YR1_HUMAN;sp Q9BRR	2	0.005382	0	0	307160	586480	0	0	0
sp O15068-3 MCF2L_HUMAN;sp O150	2	0.006801	0	0	0	225600	5781800	0	0
tr C3PTT6 C3PTT6_HUMAN;tr G8H6I3	2	0.000694	0	0	0	642848	1237300	155230	0
tr D6RFM0 D6RFM0_HUMAN;tr D6RAH	2	0	0	0	0	112390	1050500	1650600	0
sp Q7Z3Z4 PIWL4_HUMAN;tr F5H116	2	0.003311	0	197514	0	468570	3650100	56450	0
tr H3BRR0 H3BRR0_HUMAN;sp Q9NZC	1	0	18583530	2127920	2059540	2871140	4170310	5098050	0
tr Q5VXJ5 Q5VXJ5_HUMAN;tr Q9H2G	1	0.003321	14147700	16407000	11194500	9280700	26565000	19056500	0
tr B4DGK9 B4DGK9_HUMAN;sp Q9UHY	1	0	5230770	6697000	4285430	3850470	5628230	4112800	0
tr O15310 O15310_HUMAN;tr O15313	1	1	4207000	3296060	2768000	8616600	15988400	8623900	0
tr LOR6T2 LOR6T2_HUMAN	1	1	3424760	2216850	2441910	2412140	1492510	3531570	0
tr Q6ZP92 Q6ZP92_HUMAN	1	0.005198	3135400	1417740	530380	2610600	2300340	2755210	0
tr H3BS88 H3BS88_HUMAN;sp Q8N6V	1	1	3023640	6436900	5524900	4255490	9886100	8688240	0
tr C9J7L5 C9J7L5_HUMAN;tr C9JY31 C	1	0.008239	2942240	21917000	16947430	10615130	33042000	4307460	0
tr B4DJY8 B4DJY8_HUMAN	1	0.004248	2737000	0	3532000	1717800	0	0	0
tr Q499Z6 Q499Z6_HUMAN	1	1	1813100	693160	0	2141300	0	3275050	0
tr A0A024R2D7 A0A024R2D7_HUMAN;	1	0.005195	1289830	236960	818670	0	1518610	0	0



sp P01609 KV117_HUMAN	1	0	1273140	1721800	6388800	2447000	14126000	1614500
sp Q9UGI0 ZRAN1_HUMAN	1	0.006567	1208200	0	0	0	3005700	0
tr B7ZMF1 B7ZMF1_HUMAN;tr B2RTQ	1	0.003317	1038000	0	0	0	0	984160
tr B9TX32 B9TX32_HUMAN;tr B9TX33	1	0.004569	971930	385550	0	506090	2401030	0
tr B4DJB3 B4DJB3_HUMAN;sp Q8IUX7	1	1	952380	0	0	322930	0	0
tr H3BPG5 H3BPG5_HUMAN;tr H3BV8	1	0	948070	268090	1529300	0	6639100	571986
sp Q9BZG9-3 LYNX1_HUMAN;sp Q9BZ	1	0.000354	912420	854280	919540	723710	0	629250
tr Q8TEP9 Q8TEP9_HUMAN;tr A0A024	1	0.001036	901960	0	0	0	0	0
tr B4DR67 B4DR67_HUMAN;sp Q9Y67	1	0.006524	859775	0	123920	0	0	0
tr Q5T8R3 Q5T8R3_HUMAN;sp P53985	1	0.003309	823230	0	0	0	0	0
sp Q8WWL2-3 SPIR2_HUMAN;sp Q8W	1	1	813310	479870	1877400	439860	4083300	744130
tr B4DYH6 B4DYH6_HUMAN;tr B4DH8	1	0	803220	0	0	0	2079860	0
sp Q5SY68 S1A7B_HUMAN	1	0.009964	732650	0	224320	0	0	0
tr Q9P0G0 Q9P0G0_HUMAN;tr HOYI36	1	0.005435	704880	0	0	0	1217300	159190
sp P23610 F8I2_HUMAN	1	0	682220	0	0	377910	1990300	0
tr B3KNQ9 B3KNQ9_HUMAN;tr B3KW	1	0	673290	0	0	0	1037700	0
tr HOYFX4 HOYFX4_HUMAN;tr A0A024	1	0.000706	595220	0	462760	0	0	0
tr B4DH44 B4DH44_HUMAN;sp P4598	1	0.004571	594730	213870	822320	0	1555200	0
tr LOR5C7 LOR5C7_HUMAN	1	0.005404	586090	1776750	0	1020750	0	4679010
sp Q86X18 CS068_HUMAN;tr A0A087M	1	0.006818	576230	1011800	722940	934430	1049600	1338220
tr HOYLW7 HOYLW7_HUMAN;tr HOYML	1	0	574860	150890	514610	0	1782300	222420
tr F6RY50 F6RY50_HUMAN;tr B4E0W3	1	0.009979	510990	0	39593	0	0	0
sp Q9Y3R4 NEUR2_HUMAN	1	0	500460	0	0	0	0	0
sp P42224-2 STAT1_HUMAN;tr J3KPM	1	0.002697	435560	0	0	0	0	0
tr B4DMS9 B4DMS9_HUMAN;sp Q9H0	1	0	435350	0	0	0	1309600	0
sp Q9BQ50-2 TREX2_HUMAN;tr Q0657	1	0	432810	0	0	0	0	0
tr B2R717 B2R717_HUMAN;tr A0A024F	1	0.003626	429530	0	0	0	0	0
sp Q9HCD5 NCOA5_HUMAN	1	0.000355	412730	0	0	0	777790	0
tr B7Z1C2 B7Z1C2_HUMAN;tr F5H8H2	1	0	405350	0	0	0	1221400	0
tr F8VVM2 F8VVM2_HUMAN;tr Q8NC	1	0.005451	364510	0	262680	0	510740	0
tr J3KSK6 J3KSK6_HUMAN;sp Q7RTP6	1	0.00547	355780	0	0	0	0	0
tr F8WE41 F8WE41_HUMAN;sp P5229	1	0.009105	355700	0	0	0	0	537680
tr HOYMP2 HOYMP2_HUMAN;tr HOYL7	1	0.002684	346710	0	1004800	0	2218000	0
tr Q9H984 Q9H984_HUMAN;tr A0PJ59	1	0.000694	342990	0	0	0	945440	0
sp Q9Y5T5-5 UBP16_HUMAN;sp Q9Y5	1	0.000371	336180	146230	315230	0	1348900	146070
tr H3BTI5 H3BTI5_HUMAN;tr H3BVG8	1	0	330600	0	0	0	796410	0
tr B4DS19 B4DS19_HUMAN;tr B4DWW	1	0.000372	316350	0	0	0	754170	0
tr Q5HYD9 Q5HYD9_HUMAN;tr D6RFH	1	0.001035	306540	278600	389350	127170	0	0
tr A0A024R8L8 A0A024R8L8_HUMAN;s	1	0.002702	303910	0	0	0	0	0
tr H3BRX7 H3BRX7_HUMAN;sp Q1467	1	0.000371	290000	0	267810	0	0	0
tr B4DGH7 B4DGH7_HUMAN;sp Q9UUX	1	0.002719	288630	0	141230	0	647080	0
tr Q8N2P4 Q8N2P4_HUMAN;tr B3KXZ	1	0	264810	0	556000	192790	300710	0
tr F5GXG4 F5GXG4_HUMAN;sp Q96HS	1	0.00679	254820	0	401800	0	0	0
tr K7EQ71 K7EQ71_HUMAN;tr K7EKI8	1	0.005399	246360	0	0	0	226090	0
sp Q14657 LAGE3_HUMAN	1	0	239800	215090	597680	142070	3149800	312660
tr A0PJ80 A0PJ80_HUMAN;sp Q96K76	1	0.000362	232500	0	212750	0	507100	0
sp Q8WY22 BRI3B_HUMAN	1	0	218840	156640	395960	102620	327590	59466
tr B0QY86 B0QY86_HUMAN;sp Q8N3F	1	0.006552	218200	0	158770	0	0	0
tr V9HWG2 V9HWG2_HUMAN;sp Q9Y	1	1	217270	0	0	0	0	0
tr A8KAQ8 A8KAQ8_HUMAN;sp Q957	1	0.000699	208570	0	0	0	0	0
sp Q9Y446 PKP3_HUMAN;sp Q9Y446-2	1	0.005185	205740	0	0	0	0	0
tr F8WC82 F8WC82_HUMAN;tr C9J4N	1	0.000704	197100	0	0	0	1080400	0
sp Q9NVT9-2 ARMC1_HUMAN;sp Q9N	1	0.000354	183870	170270	383720	0	1246000	0
tr C9JQG7 C9JQG7_HUMAN;sp Q9Y23	1	0	176590	0	222420	0	682760	0
tr B4DDM2 B4DDM2_HUMAN;sp Q8TE	1	0.009113	173040	89738	209000	0	189280	0
sp Q9Y2S0 RPAC2_HUMAN	1	0	137000	0	362200	0	2642800	212880
sp Q60942-3 MCE1_HUMAN;tr B4DSJ8	1	0.001376	134080	0	194680	0	472200	0
tr C9J0T6 C9J0T6_HUMAN;tr H7C1P7 I	1	0.005203	132160	174680	0	223970	380460	0
tr B4DK86 B4DK86_HUMAN;tr A0A0A0	1	0	128880	0	0	0	195990	0
tr H7C3P7 H7C3P7_HUMAN;sp P11233	1	0	126570	113770	220820	0	575750	0
sp Q9P2X0 DPM3_HUMAN;tr Q86TM7	1	0	114230	0	0	0	250430	279420
sp Q7Z7F0-4 Q0907_HUMAN;tr D3DVA	1	0.000363	72552	0	118090	0	190130	32844
sp P0C2W1 FBSP1_HUMAN	1	0.005472	71195	0	113850	0	0	0
tr A0A0A0MSU2 A0A0A0MSU2_HUMAN	1	0.000357	60850	0	0	0	156590	0
tr H3BUD2 H3BUD2_HUMAN;tr H3BSO	1	0.004251	52795	0	86369	0	568240	0
tr K7EQT9 K7EQT9_HUMAN;tr K7ES75	1	0.004254	34971	0	0	0	0	0
sp Q9NPE3 NOP10_HUMAN	1	1	21204	0	54913	0	344070	0
sp O15379 HDAC3_HUMAN;sp O15379	1	0	0	0	0	0	613790	0
sp P10767 FGF6_HUMAN	1	1	0	0	271920	0	0	0
sp P16233 LIPP_HUMAN	1	1	0	0	158600	0	0	0
sp P51808 DYL73_HUMAN;tr F2Z328 F	1	0.004892	0	0	0	0	540610	0
sp P52735-3 VAV2_HUMAN;sp P52735	1	0.006589	0	0	195330	0	505380	0
sp P55081 MFAP1_HUMAN	1	0	0	0	0	0	1334500	0
sp Q07889-2 SOS1_HUMAN;tr Q8NIA3	1	0.005156	0	0	0	0	779570	0
sp Q12962 TAF10_HUMAN	1	0.000361	0	0	0	0	341500	0
sp Q15398-1 DLGP5_HUMAN;tr B4DMI	1	0	0	0	0	0	772650	0
sp Q5SYE7-2 NHSL1_HUMAN;sp Q5SY	1	0.003326	0	0	0	0	2345770	0
sp Q5T280 C1114_HUMAN	1	0.00036	0	0	0	0	1131200	0

sp Q5T2E6 CJ076_HUMAN	1	0.001716	0	0	0	0	2555200	0
sp Q5T481 RBM20_HUMAN	1	0	0	0	114420	0	919260	0
sp Q6P6C2 ALKB5_HUMAN;sp Q6P6C2	1	0.005463	0	0	0	0	1091900	0
sp Q6XZF7 DNMBP_HUMAN	1	0.001714	0	0	0	0	169750	0
sp Q7RTS3 PTF1A_HUMAN	1	0.006575	0	0	635340	0	0	0
sp Q7Z6V5-2 ADAT2_HUMAN;sp Q7Z6	1	0.007073	0	0	0	0	1270300	0
sp Q86U90 YRDC_HUMAN	1	0.000696	0	0	0	0	164980	0
sp Q86UA6 RIP_HUMAN;tr A0A0A0MS	1	0	0	0	0	0	1220900	0
sp Q86V85 GP180_HUMAN	1	0.006788	0	0	0	0	133910	0
sp Q8IXM2-2 BAP18_HUMAN;tr F8W0	1	0.006565	0	0	0	0	1061900	0
sp Q8N5L8 RP25L_HUMAN	1	0.005161	0	0	0	0	368320	0
sp Q8N9B5-2 JMY_HUMAN;sp Q8N9B5	1	0.000362	0	0	150890	0	321150	0
sp Q8N9N5-3 BANP_HUMAN;tr B3KM	1	0.007645	0	0	0	0	317750	0
sp Q8NDX6 ZN740_HUMAN	1	0.000353	0	0	0	0	782150	0
sp Q8NG68 TTL_HUMAN	1	0	0	0	791270	0	0	0
sp Q8NH76 O56B4_HUMAN	1	1	0	0	0	0	0	0
sp Q8NHU6 TDRD7_HUMAN	1	1	0	0	0	0	0	0
sp Q8TB03-3 CX038_HUMAN;tr B4E326	1	0.005419	0	0	0	0	407370	0
sp Q8TB52 FBX30_HUMAN	1	0.005376	0	0	0	0	840350	0
sp Q8TBK6-2 ZCH10_HUMAN;tr B3KVL	1	0	0	0	0	0	487610	0
sp Q8TEQ8 PIGO_HUMAN	1	1	0	0	0	0	0	0
sp Q8TF65 GIPC2_HUMAN	1	0.002692	0	0	0	0	0	0
sp Q8WUX2 CHAC2_HUMAN	1	0.005165	0	0	0	0	0	0
sp Q8WVH0 CPLX3_HUMAN	1	1	0	0	0	0	0	0
sp Q8WVY7 UBCP1_HUMAN	1	0.009132	0	0	0	0	0	0
sp Q8WXD5 GEMI6_HUMAN	1	0.00331	0	0	84293	0	667390	0
sp Q96BK5 PINX1_HUMAN	1	0	0	0	0	0	723680	0
sp Q96BN8 OTUL_HUMAN	1	0.003635	0	0	0	0	0	0
sp Q96G25-3 MED8_HUMAN;sp Q96G2	1	0.001376	0	0	0	0	410380	0
sp Q96G46-2 DUS3L_HUMAN;tr B2RDV	1	0.000365	0	0	714800	0	1292000	0
sp Q96GS4 CQO59_HUMAN	1	0.00518	0	0	0	0	1334800	0
sp Q96JP5-2 ZFP91_HUMAN;tr A0A024	1	0.000367	0	0	0	0	1508400	0
sp Q96KM6 Z512B_HUMAN	1	0.000364	0	0	0	0	1322300	0
sp Q9BQS8-3 FYCO1_HUMAN;sp Q9BC	1	0	0	0	0	0	539120	0
sp Q9BSM1-3 PCGF1_HUMAN;tr A0A0	1	0.003618	0	0	0	0	676880	0
sp Q9H8G2 CAAP1_HUMAN	1	0.005428	0	0	0	0	250680	0
sp Q9NVU7-2 SDA1_HUMAN;tr E7EW0	1	0.000695	0	0	0	0	1310321	0
sp Q9NX07-2 TSA1_HUMAN;sp Q9N	1	0.006534	0	0	0	0	399310	0
sp Q9NZM4-2 GSCR1_HUMAN;tr A0AC	1	0.006577	0	0	0	0	505340	0
sp Q9UBF6-4 RBX2_HUMAN;tr B2R4X4	1	0	0	0	0	0	383740	0
sp Q9UHR4 BI2L1_HUMAN	1	0.006556	0	0	0	0	151880	0
sp Q9Y2P8 RCL1_HUMAN	1	0.00543	0	0	0	0	596390	0
sp Q9Y4E5-2 ZN451_HUMAN;tr Q4KMI	1	0.000698	0	0	0	0	103430	0
sp Q9Y4U1 MMAC_HUMAN	1	0	0	0	0	0	1573100	0
sp Q9Y5U9 IR3IP_HUMAN	1	0.005424	0	0	0	0	1405600	0
sp Q9Y651 SOX21_HUMAN	1	0.001377	0	0	0	0	242360	0
tr A0A024QYX3 A0A024QYX3_HUMAN;	1	0.007937	0	0	0	0	935570	0
tr A0A024QZS1 A0A024QZS1_HUMAN;	1	0.006813	0	0	0	0	826230	0
tr A0A024R7R3 A0A024R7R3_HUMAN;	1	0.002695	0	0	328590	0	0	0
tr A0A024R8N4 A0A024R8N4_HUMAN;	1	0.005454	0	0	322610	0	1338870	0
tr A0A024R8Q8 A0A024R8Q8_HUMAN;	1	0.00678	0	0	205640	0	0	0
tr A0A024R9N0 A0A024R9N0_HUMAN;	1	0	0	0	182640	0	578540	0
tr A0A024RBB9 A0A024RBB9_HUMAN;	1	1	0	0	0	0	0	0
tr A0A024RCX8 A0A024RCX8_HUMAN;	1	0	0	0	0	0	587480	0
tr A0A087WTF1 A0A087WTF1_HUMAN	1	0.006528	0	0	0	0	1462260	0
tr A0A087WUD3 A0A087WUD3_HUMA	1	0.000693	0	0	0	0	740060	0
tr A0A087WUW7 A0A087WUW7_HUMA	1	0.007373	0	0	0	0	241500	0
tr A0A087WVQ2 A0A087WVQ2_HUMA	1	0.007944	0	0	89772	0	326530	0
tr A0A087WWQ9 A0A087WWQ9_HUM	1	1	0	0	0	0	0	0
tr A0A087WXF7 A0A087WXF7_HUMAN	1	0	0	0	0	0	1194600	0
tr A4D1W6 A4D1W6_HUMAN;sp Q8TA	1	0	0	0	0	0	376170	0
tr A4IF61 A4IF61_HUMAN;sp Q9POW2	1	0.009958	0	0	28649	0	768230	0
tr A6NLH6 A6NLH6_HUMAN;tr Q53HE	1	0.006782	0	0	282640	0	2016100	0
tr A8K0F7 A8K0F7_HUMAN;sp Q8N0U	1	0.0007	0	0	0	0	0	0
tr A8K0I0 A8K0I0_HUMAN;tr A0A024R	1	0.002694	0	0	0	0	433160	0
tr B0V3I0 B0V3I0_HUMAN;tr A9C4B9	1	0	0	0	0	0	146210	0
tr B1AK63 B1AK63_HUMAN;sp Q9HAF	1	0.005999	0	0	0	0	1348900	0
tr B1B5T7 B1B5T7_HUMAN;tr B1B5T0	1	1	0	0	0	0	0	0
tr B2RAX6 B2RAX6_HUMAN;sp Q9Y5J1	1	0.009096	0	0	215840	0	276130	0
tr B3KNV9 B3KNV9_HUMAN;tr S4R40	1	0	0	0	0	0	387540	0
tr B3KTC3 B3KTC3_HUMAN;sp Q9H2C	1	0	0	0	302950	0	817333	0
tr B3KV54 B3KV54_HUMAN;tr H0Y760	1	0	0	0	0	0	192440	0
tr B4DDK7 B4DDK7_HUMAN;tr B1AK6	1	0.000371	0	0	0	0	303350	0
tr B4DFK0 B4DFK0_HUMAN;tr E9PKJ2	1	0.005163	0	0	0	0	993360	0
tr B4DGW0 B4DGW0_HUMAN;sp O75	1	0.006538	0	0	0	0	706190	0
tr B4DJU5 B4DJU5_HUMAN;sp Q8IUI8-	1	0.000365	0	0	0	0	448619	0

tr B4DLZ1 B4DLZ1_HUMAN;sp Q9P107	1	0.006614	0	0	0	0	515310	0
tr B4DRB3 B4DRB3_HUMAN;tr B4DTN0	1	0.005188	0	0	0	0	479500	0
tr B4DSF4 B4DSF4_HUMAN;tr B3KS94	1	0	0	0	0	0	145510	0
tr B4DSN5 B4DSN5_HUMAN;tr A8K3M0	1	0	0	0	408320	0	0	0
tr B4DSV2 B4DSV2_HUMAN;tr B4DFD5	1	0.000362	0	0	97058	0	410230	0
tr B4DU58 B4DU58_HUMAN;sp P40121	1	0.005442	0	0	0	0	227930	0
tr B7Z821 B7Z821_HUMAN;tr A0A024F0	1	0.006602	0	0	0	0	330650	0
tr B7Z9U0 B7Z9U0_HUMAN;sp Q9UFF9	1	0.002688	0	0	0	0	621160	0
tr C4TNW4 C4TNW4_HUMAN;tr Q2VFP0	1	0.006596	0	0	0	0	344340	0
tr C9I5E0 C9I5E0_HUMAN;tr C9I2W2	1	1	0	0	386280	0	0	0
tr C9IIZ3 C9IIZ3_HUMAN;tr H7C2Z6 H	1	0	0	0	0	0	518110	0
tr C9IYM0 C9IYM0_HUMAN;sp O75811	1	0.005212	0	0	0	0	891670	0
tr C9K0U8 C9K0U8_HUMAN;tr E7EUY5	1	0.006618	0	0	132060	0	0	0
tr D6RBA9 D6RBA9_HUMAN;sp Q15281	1	0	0	0	0	0	938070	0
tr D6RC40 D6RC40_HUMAN;tr D6RCQ3	1	0.002728	0	0	0	0	187420	0
tr D6RGK9 D6RGK9_HUMAN;tr B4E2SC	1	0.00036	0	0	517380	0	0	0
tr D6RH30 D6RH30_HUMAN;tr A0A024F0	1	0.00519	0	0	0	0	2237400	0
tr E5RIV7 E5RIV7_HUMAN;tr A0A024R0	1	0.00539	0	0	0	0	2098860	0
tr E9PGE5 E9PGE5_HUMAN;sp Q9NUY0	1	0.003006	0	0	0	0	913870	0
tr E9PLD3 E9PLD3_HUMAN;tr E9PRG8	1	0.005466	0	0	0	0	1169600	0
tr E9PLLO E9PLLO_HUMAN;tr MQQZK6	1	1	0	0	111810	0	0	0
tr E9PRK7 E9PRK7_HUMAN;sp Q8N8R0	1	0.002705	0	0	0	0	716490	0
tr F8W895 F8W895_HUMAN;tr A0A0A0	1	0.008237	0	0	0	0	477770	0
tr F8WBT6 F8WBT6_HUMAN;sp Q8IXU0	1	0.002705	0	0	0	0	475770	0
tr F8WDT8 F8WDT8_HUMAN;tr G3VOC0	1	0.000364	0	0	0	0	705310	0
tr F8WEI7 F8WEI7_HUMAN;sp P09382	1	0.005423	0	0	609710	0	3181000	0
tr H0Y720 H0Y720_HUMAN;tr A0A024F0	1	0.002675	0	0	0	0	529710	0
tr H0Y9G4 H0Y9G4_HUMAN;tr H0Y9S6	1	0	0	0	0	0	420690	0
tr H0YFG1 H0YFG1_HUMAN;tr B4E3T6	1	0	0	0	0	0	1179300	0
tr H0YGN0 H0YGN0_HUMAN;tr F5GZ90	1	0.000696	0	0	0	0	659960	0
tr H0YH35 H0YH35_HUMAN	1	1	0	0	358460	0	0	0
tr H0YHB7 H0YHB7_HUMAN;sp O43341	1	0.005171	0	0	0	0	915740	0
tr H0YI13 H0YI13_HUMAN;tr J3QK86 J3	1	0	0	0	0	0	140240	0
tr H0YJ08 H0YJ08_HUMAN;tr B4E3E1 E	1	0.000359	0	0	0	0	111600	0
tr H0YLM3 H0YLM3_HUMAN;tr H0YLD4	1	0.000698	0	0	0	0	404450	0
tr H0YMN5 H0YMN5_HUMAN;tr A8K2N0	1	0.006587	0	0	0	0	1003400	0
tr H0YMR5 H0YMR5_HUMAN;tr H0YLR0	1	0	0	0	0	0	713160	0
tr H3BTU7 H3BTU7_HUMAN;sp Q9BVA0	1	0.000365	0	0	0	0	471090	0
tr H3BUU8 H3BUU8_HUMAN;sp Q08A00	1	0.00362	0	0	0	0	683430	0
tr H7C170 H7C170_HUMAN;tr Q75ME3	1	0.004258	0	0	0	0	1573400	0
tr H7C1R0 H7C1R0_HUMAN;tr B4E0G0	1	0.001037	0	0	0	0	1050000	0
tr H7C528 H7C528_HUMAN;sp Q8IXK0	1	0.006573	0	0	0	0	1635870	0
tr I3L3E4 I3L3E4_HUMAN;tr I3L4G8 I3L	1	0	0	0	140150	0	0	0
tr J3KRR0 J3KRR0_HUMAN;tr J3QQM5	1	0	0	0	0	0	198720	0
tr J3KS14 J3KS14_HUMAN;tr F5H478 F	1	0.005458	0	0	534920	0	0	0
tr J3QKK8 J3QKK8_HUMAN;sp Q8TBF2	1	0.008537	0	0	0	0	598350	0
tr J3QQW9 J3QQW9_HUMAN;tr A8K1U0	1	0.002713	0	0	278120	0	0	0
tr J7Q2I4 J7Q2I4_HUMAN;tr J7Q1C6 J	1	1	0	0	0	0	558470	0
tr K7EJ58 K7EJ58_HUMAN;tr K7EQU8	1	0	0	0	0	0	252820	0
tr K7EK72 K7EK72_HUMAN;tr K7EJIA9	1	0.008531	0	0	0	0	441860	0
tr K7EKS1 K7EKS1_HUMAN;sp P36954	1	0.00544	0	0	0	0	1045700	0
tr K7EKY2 K7EKY2_HUMAN;tr C9I258	1	0	0	0	0	0	495240	0
tr K7EN05 K7EN05_HUMAN;sp P60002	1	1	0	0	0	0	733670	0
tr L7N2F3 L7N2F3_HUMAN;tr W4VSO2	1	0.006773	0	0	0	0	100690	0
tr Q05BS8 Q05BS8_HUMAN;tr A0A0AC0	1	0	0	0	0	0	742590	0
tr Q53GY5 Q53GY5_HUMAN;sp Q8WY00	1	0.003314	0	0	0	0	673670	0
tr Q53R15 Q53R15_HUMAN;sp P05976	1	1	0	0	0	0	1383000	0
tr Q53YM3 Q53YM3_HUMAN;sp O75400	1	1	0	0	0	0	630320	0
tr Q5TBH0 Q5TBH0_HUMAN;tr Q5TBG0	1	0.000357	0	0	0	0	1640400	0
tr Q68DM7 Q68DM7_HUMAN;tr B4E020	1	0.007939	0	0	0	0	415320	0
tr Q96BL1 Q96BL1_HUMAN;sp Q96PM0	1	0.000705	0	0	0	0	209610	0
tr Q96FT4 Q96FT4_HUMAN;sp Q92733	1	0.003331	0	0	131710	0	0	0
tr Q9BRW5 Q9BRW5_HUMAN;sp Q02C00	1	0.00913	0	0	0	0	516390	0
tr Q9BUD9 Q9BUD9_HUMAN;tr E9PG40	1	0.004247	0	0	0	0	1090800	0
tr Q9UFV9 Q9UFV9_HUMAN;sp O43337	1	0.000354	0	0	234930	0	485350	0
tr Q9UHU9 Q9UHU9_HUMAN	1	1	0	0	0	0	1778100	0
tr Q9UNU2 Q9UNU2_HUMAN;tr F5GXS0	1	0	0	0	848340	0	542500	0
tr U4PP31 U4PP31_HUMAN;sp Q9P080	1	0.002679	0	0	107240	0	601020	0
tr W0FSR8 W0FSR8_HUMAN;tr F22ZT2	1	0	0	0	0	0	715120	0
tr HOYE68 HOYE68_HUMAN;sp Q9BZ95	1	0.002726	0	0	211940	0	850250	80801
tr B0I1S3 B0I1S3_HUMAN;tr K7ELN3 K	1	0.006612	0	0	0	0	2042050	201690
sp Q96EY9 ADAT3_HUMAN;tr D6W601	1	0.009116	0	0	294930	0	605350	61309
sp P18615-4 NELFE_HUMAN;tr H9ZYJ1	1	0.006608	0	0	0	0	1271000	192760
sp Q9H5X1 FA96A_HUMAN	1	1	0	0	121940	0	440660	75940
sp Q9ULR0 ISY1_HUMAN;sp Q9ULR0-2	1	0	0	0	0	0	487080	98602



tr B8ZZF8 B8ZZF8_HUMAN;tr A0A090N	1	0	0	0	0	0	0	1109000	286490
tr Q6PKD4 Q6PKD4_HUMAN;tr A8K2A	1	0.000356	0	0	0	0	0	619900	175820
tr M0QY17 M0QY17_HUMAN;sp Q9NV	1	0.009102	0	0	821480	0	0	837546	262430
tr A0A024R829 A0A024R829_HUMAN;s	1	0	0	0	0	0	0	814390	255890
tr B2R4N3 B2R4N3_HUMAN;tr A0A024	1	0.000696	0	0	389910	0	0	1151000	464230
tr E9PS71 E9PS71_HUMAN;tr B7Z8B3	1	0.002704	0	0	0	0	0	457790	200248
tr B2R5H5 B2R5H5_HUMAN;sp P62310	1	0.005465	0	0	0	0	0	1439500	776820
tr F8W099 F8W099_HUMAN;tr F8W60	1	0.002733	0	0	0	0	0	278440	219850
sp P36406-3 TRI23_HUMAN;sp P36406	1	0.002698	0	584670	0	0	0	1980400	0
sp Q4G0P3-2 HYDIN_HUMAN;sp Q4GC	1	1	0	37778	0	0	0	0	0
tr A0A024R978 A0A024R978_HUMAN;s	1	1	0	318700	0	0	0	0	0
tr F8VS92 F8VS92_HUMAN;tr F5H023	1	1	0	131430	0	0	0	0	0
tr I7H6J7 I7H6J7_HUMAN	1	1	0	292300	0	0	0	0	0
tr Q8N9Z3 Q8N9Z3_HUMAN;sp Q1293	1	0.007369	0	189350	579380	0	0	0	0
tr B3KN59 B3KN59_HUMAN;tr B3KM3	1	0	0	256430	203140	0	0	1162400	378620
tr S4R460 S4R460_HUMAN;tr A0A087V	1	0	0	0	872230	169240	0	0	425960
sp Q9GZT9-2 EGLN1_HUMAN;tr R4SCC	1	0	0	144880	703870	153370	1044800	184630	0
tr B4DEN5 B4DEN5_HUMAN;tr B3KQQ	1	0.000366	0	182920	355860	110140	0	0	0
tr Q5T4U8 Q5T4U8_HUMAN;tr Q59GT	1	0.00036	0	0	790410	264080	1360800	253860	0
tr B4DQC9 B4DQC9_HUMAN;sp Q9BX	1	0	0	0	506390	169440	1106600	0	0
tr H0YL59 H0YL59_HUMAN;sp Q9BYU1	1	0.009943	0	0	1205700	676880	665840	0	0
tr M1FWY0 M1FWY0_HUMAN	1	0.00268	0	2780600	2963900	2238400	9965300	0	0
sp P01602 KV110_HUMAN	1	0.002723	0	2878300	156510	438500	1021300	4401300	0
sp Q5VTQ0-7 TT39B_HUMAN;sp Q5VT	1	0.001037	0	363692	0	813310	988580	761180	0
tr A2J1N8 A2J1N8_HUMAN	1	0.006593	0	9039030	0	8768120	771020	13553650	0